	Inventaris Wob-verzoek W16-22S								
		wordt verstrekt				**weigeringsgronden**			
nr.	**document**	**reeds openbaar**	**niet**	**geheel**	**deels**	**10.1.c**	**10.2.e**	**10.2.g**	**11.1**
	NTS2016407								
1	Aanvraagformulier				x		x	x	
2	Projectvoorstel			x					
3	Niet-technische samenvatting	x							
4	Bijlage beschrijving dierproeven 1			x					
5	Bijlage beschrijving dierproeven 2			x					
6	Bijlage beschrijving dierproeven 3			x					
7	Bijlage beschrijving dierproeven 4			x					
8	Bijlage beschrijving dierproeven 5			x					
9	DEC-advies				x		x	x	
10	Ontvangstbevestiging				x		x	x	
11	Advies CCD		x						x
12	Beschikking en vergunning				x		x	x	

29 JAN. 2016

AVD 301002016407

Centrale Commissie Dierproeven

Aanvraag
Projectvergunning Dierproeven
Administratieve gegevens

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

1 Gegevens aanvrager

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

☒ Ja > *Vul uw deelnemernummer in*	30100
☐ Nee > *U kunt geen aanvraag doen*	

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

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

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

1.4 Vul de gegevens in van de verantwoordelijke onderzoeker.

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

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

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

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

2 Over uw aanvraag

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

3 Over uw project

3.1	Wat is de geplande start- en einddatum van het project?	Startdatum	15 - 2 - 2016
		Einddatum	15 - 2 - 2021
3.2	Wat is de titel van het project?	Pre-clinical intervention studies in mice for prevention and treatment of cancer	
3.3	Wat is de titel van de niet-technische samenvatting?	Preklinische interventiestudies in muizen voor kankerpreventie of behandeling	
3.4	Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?	Naam DEC	NKI
		Postadres	t.a.v. [redacted] Postbus 90203;1006 BE; Amsterdam
		E-mailadres	[redacted]

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

☒ Nieuwe aanvraag Projectvergunning € 1727,- Lege

☐ Wijziging € Lege

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

☐ Via een eenmalige incasso

☒ Na ontvangst van de factuur

5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht

☒ Projectvoorstel

☒ Niet-technische samenvatting

Overige bijlagen, indien van toepassing

☐ Melding Machtiging

☒

6 Ondertekening

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

Centrale Commissie
Dierproeven
Postbus 20401
2500 EK Den Haag

Ondertekening door de instellingsvergunninghouder of gemachtigde (zie 1.7). De ondergetekende verklaart:

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

Naam	[redacted]
Functie	[redacted]
Plaats	Amsterdam
Datum	27 - 1 - 2016
Handtekening	[redacted]

Form
Project proposal

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

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	30100
1.2	Provide the name of the licenced establishment.	Stichting Het Nederlands Kanker Instituut – Antoni van Leeuwenhoek ziekenhuis
1.3	Provide the title of the project.	Pre-clinical intervention studies in mice for prevention and treatment of cancer.

2 Categories

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

- ☒ Basic research
- ☒ Translational or applied research
- ☐ Regulatory use or routine production
- ☐ Research into environmental protection in the interest of human or animal health or welfare
- ☐ Research aimed at preserving the species subjected to procedures
- ☐ Higher education or training
- ☐ Forensic enquiries

☐ Maintenance of colonies of genetically altered animals not used in other animal procedures

3 General description of the project

3.1 Background

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

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

Cancer is a major cause of death in the western world. In the Netherlands, it is estimated that there will be roughly about 123,000 new cases diagnosed annually around the year 2020, resulting an overall mortality of 50,000 patients/year (source: ISBN 978-90-71229-00-8; Kanker in Nederland tot 2020: Trends en Prognoses; KWF Kankerbestrijding, September 2011). The burden to the society associated with cancer, both in terms of suffering of patients and relatives as well as socio-economic impact is substantial.

Cancer is a disease that is characterized by uncontrolled growth, due to genetic and epigenetic alterations that disrupt the balance between cell proliferation, stasis and cell death. An important characteristic of malignant tumors is the capacity of forming metastases. Importantly, the dissemination of tumor cells from the primary tumor can occur at an early stage in the disease, in many cases before the diagnosis has been made. As a consequence, surgical resection of the primary tumor alone is insufficient to cure the patient and adjuvant treatment (radiotherapy, chemotherapy, hormonal therapy, immunotherapy) is necessary.

Cancer comprises of a collection of diseases, traditionally sub-divided on the basis of the anatomical location, histology and immunocytochemistry. This traditional classification was (and still is) important in the design of a treatment plan. However, during the last two decades of ongoing research, the molecular pathology underlying the process of malignant transformation is being unraveled in great detail. Similarly, the impact of the stromal component and the immune system on tumor and metastases formation are much better understood. Importantly, this newly obtained knowledge has now started to contribute to the development of improved therapies for cancer patients. Examples are the development of trastuzumab, a humanized monoclonal antibody directed against Her2, a receptor driving tumor cell proliferation in a subset of breast cancer patients (Hudis, N Engl J Med (2007) 377: 39-51), imatinib (Glivec) a small molecule inhibitor of Bcr/Abl for treatment of chronic myeloid leukemia (Stegmeier et al, Clin Pharm Ther (2010) 87: 543-552) and vemurafenib an inhibitor of BRAF-V600E for treatment of a subset of melanoma patients (Bollag et al, Nature (2010) 467: 596-599). Moreover, for melanoma there are also exciting improvements based on novel immunotherapies (Couzin-Frankel, Science (2013) 342: 1432-1433). All such improvements are the fruit of the collaborative efforts of the cancer community (scientist and clinicians). However, given the high number of potential cancer drug targets and the highly complex problem of acquired therapy resistance, there are many more efforts required to offer the prospect of cure or long-term disease control with acceptable side effects to the majority of cancer patients. The success stories with the targeted agents as mentioned above are due to the fact that these tumors are primarily driven by one specific oncogenic alteration that can be targeted specifically by a drug. Many cancers, however, are the product of several mutated/activated pathways working in concert. Moreover, also in case of the above-mentioned successes, the emergence of resistance due to adaptive power of the cancer cell by rewiring of the oncogenic signal transduction pathways makes that responses to these new treatment can be relatively short-lived. Consequently, the successful treatment of such tumors will depend on an integrative approach that may involve radiotherapy, combinations of anti-cancer therapeutics and immune therapy. An important paradigm shift is that the traditional sub-classification of tumors (anatomical location) may no longer be the most appropriate instrument to determine the treatment schedule, but actually the underlying molecular pathology is becoming key in choosing the optimal treatment option. For example, the PI3k-Akt-mTOR pathway is activated in many tumor types and there are many drugs that target this pathway in the pipeline. The potential utility of such drugs will likely rely on the dependence of the tumor on this pathway, rather than on its original location (lung, breast, brain cancer, etcetera).

The mission of Netherlands Cancer Institute is to reduce the incidence of cancer and to improve the treatment of cancer patients by performing fundamental and applied / translational research aimed at getting a better understanding of the processes underlying tumorigenesis, progression, therapy and resistance. For this purpose, the institute accommodates specific research groups working on tumor biology, molecular biology, radiation biology, immunology, pharmacology and nuclear medicine. The development and introduction of new technologies and therapeutics into the clinic is one of the spearheads of our institute. Next to conducting clinical studies, the development and implementation of preclinical models involving laboratory animals is an essential component of the overall research program that is required to develop better cancer treatments. Furthermore, fundamental research provides in-depth information and new insights in potential new drug targets the cancer cell. On the other hand, there are numerous possible combination of therapies that may

be of potentially use, but it is ethically unacceptable and practically impossible to test all treatments and combinations thereof immediately in patients. Before moving investigational (combinations of) therapeutics to the clinical stage of development, preclinical studies in relevant disease models need to be conducted to provide information about soundness of the strategy, the best candidate agents and drug exposures required for target inhibition and the most optimal combinations of treatments. For this purpose the NKI has received an NWO roadmap grant. This program of roadmap grant includes the development of new transgenic animal facility to develop state-of-the art techniques for genetic modifications and to use these models for studying the tumor biology. These parts of the program will be addressed in other CCD projects. Part of the NWO program is to realize a dedicated mouse cancer clinic (MCC) with facilities that mirror the clinical facilities in our cancer hospital, so that all the relevant clinical modalities can be integrated in our preclinical research.

This project comprises all the studies and animal handlings that will be performed within the framework of this mouse cancer clinic and will include the following work packages: Disease induction, pharmacokinetics/pharmacodynamics (PK-PD), preventive and therapeutic interventions. In the NKI, we have developed a range of mouse cancer models of various tumor types that recapitulate many aspects of the human disease. These include tumor cell line models, transgenic mouse models and patient-derived xenografts models. Moreover, we are continuously improving these models (work that will be performed under separate CCD projects as mentioned above). The "patients" in the mouse cancer clinic will be mice bearing such tumors that are relevant to the corresponding human disease and/or therapies that we want to study. We have available or will develop (under a separate CCD project license) non- or minimally-invasive techniques to visualize tumors for diagnosis, proliferation, delivery of therapeutics and for assessment of therapy response that are based on anatomic (CT, MRI), optical (bioluminescence, fluorescence), acoustic and nuclear (SPECT, PET) imaging. A CT image-guided small animal cone beam irradiator is operational that can be used for precise delivery of narrow beams (down to 0.5 mm) of radiation at multiple angles, similar to stereotactic radiotherapy in patients. Our mouse pharmacy unit provides access to the relevant new agents (small molecule drugs, antibodies, other biologicals) for (targeted) therapies. These critical studies will also involve pharmacokinetics and pharmacodynamics measurements studies in order to confirm/demonstrate that the used intervention is efficacious in terms of target engagement.

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 this project is to assess feasibility of novel therapeutic interventions in terms of their efficacy and tolerability and to translate these findings to enable further studies in cancer patients. Obviously, extending the survival of all cancer patients is a long term goal that will not be achieved by the end of the 5 year period of this project. Nevertheless, we expect that the work performed under this project will have contributed to significant improvements in some cancer therapies.
The Netherlands Cancer Institute houses a range of research groups working on different aspects of cancer and cancer therapeutics. At some point, many of these groups will need to use in vivo studies to investigate the potential efficacy of their therapeutic concept. Although the various research groups approach cancer therapy from different angles (e.g. immunotherapy, radiotherapy, DNA repair) there is substantial overlap in the agents and modalities that are needed. For example, radiotherapy may boost immunotherapy by the creation of radiation-induced neo-antigens. Likewise, targeted therapies are tested in conjunction with immunotherapy and radiotherapy. In order to conduct these animal studies in the most efficient way, the Netherlands Cancer Institute has installed a dedicated team to supervise and/or execute such studies, instead that each research group will design and conduct preclinical efficacy studies on their own. By this centralized setup we will create a central database that ensures optimal exchange and/or re-use of results from previous studies between research groups. In line with the fact that there is no 'magic bullet' for cancer, the work performed in this project will become a collection of smaller-sized studies to interrogate the merits of many different therapies and combinations thereof in relevant disease models. The results of these studies will render pivotal information on the usefulness of the particular therapy and/or combinations of therapies under study in subsequent clinical trials. Vice versa, the results will also provide information on the validity of the target that is studied and the need for further adaptations in the concept that is used.

These objectives can be achieved since on the one hand, the NKI holds the cancer models and expertise to conduct such studies, whereas on the other hand, there are close collaborations between the clinical oncologists and the involved research groups. The mouse cancer clinic holds a (growing) collection of advanced cancer models that are well-characterized. These include genetically engineered mouse models (GEMM) that develop tumors spontaneously due to the presence of conditional alleles of oncogenes and tumor suppressor genes that can be activated/inactivated by tissue-specific promotors driving expression of Cre recombinase, (*e.g.* WAP-Cre;EcadF;P53 for invasive lobular breast carcinoma) or following somatic induction (e.g. by tamoxifen for CreERT driven tumors or adenoviral Cre inhalation for lung tumors). These model systems provided and will provide a tumor bio-bank of tumor biopsies/pieces and established cell lines that can be used for efficacy testing after transplantation into syngeneic recipient animals. Similarly, the MCC has a growing collection of patient derived xenografts; tissue pieces/biopsies from patient tumors that are transplanted and expanded in immune compromised mice to maintain as good as possible the original genotypic/phenotypic characteristics of the tumor as well as a collection of established human tumor cell lines that are commonly used for efficacy testing by the cancer community. Moreover, the NKI has the required infrastructure to conduct these studies: The MCC offers a negative barrier in order to allow hosting of mice that originate from outside the facility. The studies will be performed and/or supervised within the framework of the intervention unit by a dedicated well trained staff. The facility is comprised of a state-of-the-art animal facility with all mice being housed under SPF conditions, that disposes of all the necessary imaging modalities, a mouse pharmacy unit and a dedicated animal pathology department with two animal pathologists.

3.3 Relevance

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

Scientific: We are now in the era, where we are making large steps in understanding the process of cancer formation and progression and are identifying many potential drugable targets that may be exploited for the benefit of cancer patients. It is of pivotal importance that the usefulness of these potential targets is validated in specific disease models in order to design good clinical studies. The qualities of our mouse models of cancer will directly contribute to this preclinical – clinical translation and should ultimately help to reduce the high attrition rate of oncology drugs (currently 95 of 100 drugs entering into Phase I clinical trial are abandoned during Phase II/III trials.
Social: Health and well-being are generally considered to be of primary value by the population. Cancer is a factor that has a very negative impact on health / well-being of many people, not only patients but also their relatives and friends. Moreover, the loss of lives and morbidity also has considerable negative economic consequences (high cost of care, loss of productivity). This project, improving cancer therapy, reducing morbidity and mortality and adverse effects will first of all be of importance to the patients that will benefit from these therapies.

3.4 Research strategy

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

The Netherlands Cancer Institute houses a range of research groups working on different aspects of cancer and cancer therapeutics. Many of these groups will need to use in vivo studies to investigate the potential efficacy of their therapeutic strategies. As described above, these studies will be conducted under the auspices of the intervention unit. The setup of these studies will be very similar in structure as outlined below (3.4.2) in the 5 common protocols. Integration of these studies will direct the design of new studies to establish a pipeline that will ultimately result in a rich database comprising information that allows us to propose novel clinical treatment options.
The smaller-sized studies can be initiated by results obtained by fundamental or clinical research. To list just a few examples: Fundamental research may provide /reveal potential targets or a novel resistance mechanism that needs to be validated in vivo before going to the patient (*i.e.* bench-to-bed side approach). An example of this is the finding that Brca1 deficiency (a trait of a subset of (inheritable) breast cancers disrupting the homology-directed DNA repair pathway in cells) makes tumor cells more vulnerable to drugs targeting other DNA repair pathways, viz. PARP inhibitors, as a consequence of synthetic lethality. This concept has first been discovered in vitro, next validated in in vivo models and is now being exploited in cancer patients (see for example Clintrials.gov: NCT02032823). On the other end, observations made in the clinic can also trigger preclinical research that will subsequently lead to results that will move back to the clinic (*i.e.* bedside-to-bench-to-bedside approach). An example is the discovery why colon cancers in general do not respond to the BRAF-V600E inhibitor vemurafenib, although a subset of these tumors does contain this activating mutation. It was shown by *in vitro* tests that resistance was due to a feed-back loop causing activation of the receptor tyrosine kinase EGFR (bedside-to-bench). Concomitant use of both vemurafenib and an EGFR inhibitor was able to block proliferation in vitro and in vivo, and this finding is now further explored in clinical trials (back to bedside)(Sun et al. Nature 2014;508:118-122; Clintrials.gov: NCT01791309). Another scenario is that a new candidate agent or pharmaceutical product for a particular target is discovered / developed by us or a collaborator needs to be tested for efficacy in vivo. An example is the development of a glutathione conjugated liposome carrying doxorubicin for enhanced delivery to brain tumors that is now in clinical trial (Gaillard et al, Proceedings AACR; 2014; CT216). We will select the appropriate disease models for the intervention(s) that we want to study as further outlined in section 3.4.2.
In general we will need to determine the tolerability of the intervention procedures in our specific models. Importantly, when using agents (small molecule drugs or other) we will not (only) focus on the maximum tolerated doses (MTD), but will investigate the relationship between systemic drug exposure and response. For this purpose, supportive pharmacokinetics studies will be conducted. This information will provide information on the drug plasma levels in patients that are needed in order to be potentially efficacious. Besides monitoring efficacy (effect on tumor growth), we also need to conduct studies to establish whether the pharmacological target is reached by supportive pharmacokinetic-pharmacodynamics studies. Non- or minimally-invasive imaging techniques are present and new ones will be developed under a separate CCD project license, and will be utilized for longitudinal follow up of tumor burden (disease progression / response) and/or for visualization of biological response by interrogating target engagement.

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.

We have designed a decision-tree (go/no-go moments) according to which the preclinical intervention studies will be conducted.

A new study will start either from 1a or 1b.

1a. Testing new agents (first-in-mouse study):
An agent that has not been tested previously in mice can be considered for in vivo testing when:
-It has demonstrated activity *in vitro*. This can either be direct (cytotoxicity) or indirect (e.g. target inhibition/modulation).
Or:
-There is a clear rationale for a new agent but its action cannot be validated in vitro (e.g. some immunomodulatory effects, or compounds that need in vivo bio-activation).
And:
-The stability of the compound is sufficient and can be prepared in a suitable formulation for *in vivo* studies.

-For small molecule drugs, we will first establish systemic exposure.
Compounds will be administered using a parenteral route (i.v. or i.p.) at an expected safe (non-toxic) dose to assess systemic exposure / metabolic stability.
-If intolerable adverse reactions are observed at the test dose, we will consider using a lower dose level or reject the agent for further testing.
-If metabolic stability is considered to be insufficient, we will halt further *in vivo* testing, otherwise we will continue to step 2.

1b. Testing known agents (previously tested in mice) or treatment modalities in new combinations:
In general, known agents will be tested in combinations with other agents or treatment modalities (radiotherapy, immunotherapy). Such combinations can be considered when:
-*in vitro* data supporting this combination is available. This can either be direct (cytotoxicity) or indirect (e.g. target inhibition/modulation).
-There is a clear rationale for a combination that cannot be validated *in vitro* (e.g. immunomodulatory effects).

2. Perform an MTD study to find the dose, route and schedule that can be safely administered during the intervention study.
-For small molecule drugs: We will halt further in vivo study if the safe dose is not expected to provide sufficient systemic exposure for efficacy. Otherwise we will continue to step 3

3. Select appropriate model(s) for intervention study (see also table 1 below)
The selection criteria are:
In order to be relevant, the tumor model selected for the intervention study should carry the appropriate target. This can be, for example, an activating mutation that would be the aim of a particular targeted therapeutic, but can also be a certain tumor genotype that makes the tumor more vulnerable to a specific therapy (e.g. Brca deletion and alkylating agents or PARP inhibitors). Usually, the first in vivo tests will be done using the same cell lines that have been used during the prior enabling in vitro studies. These initial tests will include efficacy studies and pharmacokinetics-pharmacodynamics studies. If these initial *in vivo* tests are unsuccessful, the investigational intervention will be discontinued. When successful, it will in general be necessary to perform confirmatory studies in other models, such as the PDX models or the transgenic models. Confirmatory studies may also include models lacking the appropriate target (as a negative control) to understand/confirm the mechanism of action.

If no previous in vivo data is present (from our own work or from the literature), we will start with a relevant model (as outlined above) that has the least possible level of discomfort. If good quality data is available (e.g. from the literature), we will build upon this information and may select a more stringent (complex) model to begin with. In order to minimize the amount of ineligible study animals, we will select the tumor model with a high take rate and consistent growth characteristics, provided it characteristics are compatible with the intervention that we would like to validate. Usually we will start with a ectopic (subcutaneous) model (Table 1a).
If the location of the tumor is relevant (e.g. brain tumors and the blood-brain barrier), we will select an orthotopic model (Table 1b).
Tumor models with less favorable growth/take rate (e.g. certain PDX models) and GEMM models will only be selected when they have unique features that are essential to test the investigational therapy.
If the intervention is aimed for treating metastases, a relevant metastatic model will be chosen. Metastatic models from cell lines injected into the blood stream are most robust, but cannot be used when dissemination from the primary tumor is a target of the intervention. Metastases from transplantable models are used for such purposes. Metastases from GEMMs are required when the intervention also targets early events in the metastasic process. For example targeting neutrophil priming of tissues that precedes metastases formation (Coffelt et al, Nature 2015; 552: 345-8).

Table 1. Overview of available in vivo tumor models

1a. Primary ectopic tumor models

Tumor source	**Origin**	**Host**	**Location of grafting**	**Metastatic capacity**	**Discomfort[1]**	**Take rate / consistency[2]**	**Remarks**
Cell line[3]	Mouse	Syngeneic	subcutaneous	Low to none	Mild to moderate	High, >80%[4]	In general low discomfort, unless ulcerating tumor occur (=humane endpoint)
Cell line	Human	Immune deficient		Low to none		High, >80%	
Patient Derived Xenograft (PDX)	Human	Immune deficient		Low to none		Variable, >50%	

1b. Primary orthotopic tumor models

Tumor source	**Origin**	**Host**	**Location of grafting**	**Metastatic capacity**	**Discomfort[1]**	**Take rate / consistency**	**Remarks**
Cell line[3]	Mouse	Syngeneic	Depends on origin of the tumor	Moderate/high	Mild to severe, depending on location	High, >80%	In general, it will be the primary tumor site that determines discomfort and not the metastases
Cell line	Human	Immune deficient		Moderate/high		High, >80%	
Patient Derived Xenograft (PDX)	Human	Immune deficient		Possible		Variable, 50%	

[1] When grown until humane endpoint is reached
[2] Consistency: The tumor needs to reach a predefined size within a predefined time window for inclusion into a study cohort.
[3] Cell line can either involve injection of a cell suspension or implantation of tissue fragments
[4] Expected average percentage of eligible animals for intervention studies

Table 1 continued

2. Spontaneous or inducible Transgenic models (GEMMs)[5]

Location	Tumor Type	Host	Induction	Metastatic capacity	Discomfort[1]	Take rate / consistency	Remarks
Brain	Glioblastoma	Inducible	lentiviral-Cre	none	Severe	High, 70-90%	Locally invasive, neurologic symptoms
Breast	Ductal carcinoma (DC) based on Brca loss	Spontaneous inducible	K14Cre/WAPCre/ lenti-or adenoviral-Cre	Low	Mild/Moderate	High, 60-90%	About 30% of KCre will develop skin tumors and cannot be used
Breast	Invasive Lobular carcinoma (ILC) based on Ecad loss	Spontaneous Inducible	K14Cre/WAPCre/ lenti-or adenoviral-Cre	Moderate/high	Mild/Moderate	High, >60-80%	About 30% of KCre will develop skin tumors and cannot be used.
Breast	ILC models based on PI3K/Akt mutations	Spontaneous Inducible	K14Cre/WAPCre/ lenti-or adenoviral-Cre	Low	Mild/Moderate	High >80%	Locally invasive. About 30% of KCre will develop skin tumors and cannot be used.
Breast	Her2+ BC	Spontaneous	MMTV-NeuT	Low	Mild/Moderate	High >80%	
Lung	NSCLC	Inducible	adenoviral-Cre	Low	Moderate/Severe	High, >70-90%	Breathing distress at end stage
	SCLC	Inducible	adenoviral-Cre	Moderate/high	Moderate/Severe	High, >70-90%	Ditto, Long lag time (8 months)
	Mesothelioma	Inducible	adenoviral-Cre	Moderate	Moderate/Severe	High, >70-90%	Breathing distress at end stage
Skin	Melanoma	Inducible	Cre-ERT/Tamoxifen	Low/moderate	Mild/Moderate	Medium, 40-60%	50% develop other skin tumors

3. Metastasis models

a. Any of the above mentioned models, where the primary tumor will be resected before tumor burden reaches the humane endpoint

Location, tumor type, host and induction	Metastatic capacity	Discomfort[1]	Take rate / consistency	Remarks
Model dependent	Sufficient	Mild/moderate	Variable	

b. Cell suspensions injected directly into the blood stream and forming lesions at (a) distant site(s)

Metastatic site	Site of injection	Handling				
Lung	Tail vein	Easy		Severe	High, >70-90%	
Liver	Spleen (+ subsequent splenectomy)	Moderate		Severe	High, >70-90%	
Brain	carotid artery	Complex		Severe	High, >70-90%	
General	Cardiac (left ventricle)	Moderate		Severe	Moderate, >50%	

[5] Other GEMMSs are under development (under different DEC/CCD projects) and may be used for intervention studies when well characterized

4. Assessment of the efficacy of the treatment

The efficacy of the therapeutic intervention can be judged by assessing the effect on the growth of the tumor and overall survival (a) or efficacy can be deduced from the capacity of the therapy to modulate the target by studying target inhibition and pharmacokinetic / Pharmacodynamics (PK/PD) relationships) (b). Efficacy and PK/PD studies can be performed simultaneously or sequentially. The order of the experiments (described below in *a* and *b*) will be determined by the research question and the availability of useful PD markers. Establishing efficacy is usually the goal of our studies and may be the starting point, but PK/PD studies can be necessary as separate studies in case we need to interrogate whether the modulation of a certain pharmacological target can be achieved, but that modulation of this target may not in itself have a direct effect on tumor growth or survival.

***a*. Treatment efficacy judged on tumor volume / survival**

-Perform tumor induction. Use appropriate surgical techniques and pain control as necessary.
-Build study cohorts from animals that have a tumor of a predefined size in a predefined time window. (Remove remaining animals or when possible use for other purposes, e.g. PK/PD study, or imaging studies).
-Perform the treatment(s) of the study cohorts
-Monitor tumor growth by appropriate methods (caliper, imaging, blood values of soluble luciferase).
-Score animal health status (body weight, movement, grooming).
-Kill the animal when the humane endpoint is reached, score survival, tumor size, metastatic burden and/or spectrum of metastases.

***b*. Treatment efficacy judged on PK / PD relationship**

Same procedures as performed in 4a. However, in this case the tumors will be allowed to grow to a size that is large enough to obtain sufficient material for PK/PD studies (as detailed in appendix 5). PK/PD studies require sampling of blood and/or tissues. In general, this will be some time before the humane endpoint has been reached and thus the level of discomfort due to tumor burden will be less. Alternatively, non-invasive imaging techniques may be developed / available to monitor PD parameters (development of imaging techniques will be performed under a separate CCD license). Such non-invasive techniques can be done at multiple times and may help to reduce the amount of animals needed per study.

5. Study evaluation

Subsequent tests will depend on the study results.
If the study has been conducted technically satisfactory and the outcome is conclusive and negative, the study will be finished.
If the study results are not conclusive but warrant further exploration, we will redesign and repeat the experiment when such improvements are feasible and a second attempt is deemed to be more successful. If not, the studies with this intervention will be halted.
If the study has been conducted technically satisfactory and the outcome positive, the study may be complete, although in general, efficacy will need to be confirmed in a second (usually more stringent) relevant model.
If the experimental treatment is efficacious, indicating that the concept of the proposed intervention may be valid, further testing may be needed to the support the translation from experimental mouse models to clinical studies in patients. This may involve testing at adapted dose levels or dose schedules.

Table 2. Overview of animal numbers and estimated level of discomfort (numbers are for a 5 year project)				
Appendix number (see below)	**Type**	**#animals**		**% at risk**
1. Interventions using transplantable tumors	Interventions	24,375	Mild	35
			Moderate	55
			Severe	10
	Model consistency check	2,000	Mild	60
			Moderate	35
			Severe	5
2. Interventions using GEMM mice	Interventions	8,750	Mild	50
			Moderate	25
			Severe	25
3. Interventions using metastatic models	Interventions	4,900	Mild	25
			Moderate	25
			Severe	50
	Model consistency check	250	Mild	25
			Moderate	25
			Severe	50
4. Tolerability studies	MTD	5,000	Mild	50
			Moderate	30
			Severe	20
5. Pharmacological studies	PK/PD	10,000	Mild	80
			Moderate	19
			Severe	1
Grand total		55,275	Mild	47
			Moderate	38
			Severe	15

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.

As mentioned above, this project clusters the activities around tumor intervention studies conducted at the NKI-AvL. Although the particular interventions can be diverse, all studies are very similar in experimental design and structure. By nature, these are more or less independent smaller-sized studies that are clustered around the central theme to develop better therapies for cancer patients. The coherence of steps taken within a study has been outlined under 3.4.2 above.
New intervention studies will always be started under this CCD project.
Ongoing intervention studies that are currenty being performed under approved DEC projects are mostly of relative short duration and will therefore not be (re)placed under the here presented CCD project. If amendments to these DEC projects are requested, these will be evaluated by the IVD. If the

amendment is only minor the IvD may decide that the experiment can be continued under the existing DEC project, otherwise the investigator will need to submit a new work protocol that should be appropriate to run under the present CCD project.

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	Intervention protocol for recipient mice with transplantable tumors
2	Intervention protocol for genetically engineered mice with spontaneous or somatically induced tumors
3	Intervention protocol for recipient mice with metastatic tumors
4	Tolerability studies
5	Pharmacological studies
6	
7	
8	
9	
10	

Appendix
Description animal procedures

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

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	30100
1.2	Provide the name of the licenced establishment.	Stichting Het Nederlands Kanker Instituut – Antoni van Leeuwenhoek ziekenhuis
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 01 — Type of animal procedure: Intervention protocol for recipient mice with transplantable tumors

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

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

This appendix describes the use of transplantable tumor models in mice as a surrogate for cancer patients to validate targets that can be used as anti-cancer therapy and/or can be used to circumvent host-related or tumor-related factors that may cause therapy resistance. To this end, tumor-bearing mice will be used to assess the antitumor effects of pharmacological, radio-therapeutic and/or genetic interventions. This appendix describes the use of mice in which a cell line or tumor suspension or a tumor piece is ectopically or orthotopically injected/implanted. The selection of the specific tumor type for transplantation

will be based on the type and/or genetic abnormalities of the tumors in patients that we want to mimic. After implantation the cells will form tumors. The growth and behavior of these tumors will be monitored by appropriate techniques and animals will be treated by the investigational treatment. The readout can be based on tumor progression (visual or imaging), survival (humane endpoints) and/or biological effect (based on analysis following the resection/collection of relevant tissue material). The aim of these experiments is to develop better treatments and/or obtain results that may be applied / translated to the clinic.
In general these transplantable tumor models are reproducible and robust in terms of tumor take rate and growth consistency.
The transplantable tumor models are usually used as the first in vivo model to interrogate the efficacy of the experimental therapy and will use the same tumor model (cell line) as was used in the prior in vitro studies).

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

1: Transplantation

1a: Ectopic transplantation
Induction of tumors via subcutaneous injection of tumor cell suspensions of a cell line or subcutaneous engraftment of a tumor fragment into an animal. The applications will be performed under appropriate anesthesia and analgesia .

1b: Orthotopic transplantation
Implantation of tumors in animals via local injection/application of tumor cells/pieces. The site of tumor implantation depends on the model that is required to mimic the specific cancer in patients. For example we will use mammary fatpad implantation of breast cancer tumors, intra-tracheal instillation of tumor cell suspensions for modelling lung cancer or inject tumor cell suspension intra-cranially into recipient mice to mimic brain tumors. All applications will be performed under appropriate anesthesia and analgesia as required per procedure.

As a result of the transplantation procedures (orthotopic or ectopic), tumors will develop, but time of onset and speed of growth will vary per model. In general, models with a high tumor take rate and consistent tumor growth will be used. A tumor model that has less optimal take rate and consistency may be used, but only in such cases where a tumor model has unique features that are not available in other models. For example, there are just a few human Her2 expressing breast cancer cell lines that are available, but most of them have a relatively poor tumor take.
All tumor models are already available or will be developed and characterized under a separate CCD project license. However, when such models, such as existing cell lines from the ATCC or other collaborators are introduced into our animal facility, it will be necessary to run a pilot test to determine/confirm the tumor take rate and growth consistency.

Animals will be monitored using appropriate techniques and at a frequency that is appropriate for the model. The discomfort caused by the tumor will depend on the model. In general, mild to moderate for more superficial tumor such as skin and mammary tumors that do not cause symptomatic metastases. The discomfort caused by tumors growing inside internal organs can be severe when grown to a size that the humane endpoints are reached (e.g. loss of body weight >20% of the initial weight, abnormal breathing, abnormal posture, etc.).

2: Tumor development and progression follow-up

In case of superficial tumors we can use palpation and/or caliper measurement to measure tumor growth. To this end, the mouse will be fixed by hand and the tumor palpated. Tumors larger than about 3 mm will be measured by calipers. Mild discomfort.

In cases of follow up of tumor volume when tumors are not superficial and/or to obtain biological information from the tumor (growing superficial and inside internal organ sites), we will need to use appropriate imaging techniques (IVIS, SPECT, MRI, PET, CT and ultrasound). Examples are to monitor distribution of a radiolabeled antibody or to measure apoptosis by ^{99m}TC-Annexin. As part of the imaging procedure, the injection of a radioactive tracer (for PET and SPECT) or contrasting agent (MRI, CT, ultrasound) may be used. If not yet available, these imaging procedures will be developed and validated under a separate CCD project license. In some cases, tumor cells have been transduced with soluble luciferase and blood sampling (25 ul from the tail) can then be used to monitor tumor growth. Mild discomfort.

Duration and frequency varies between 5-10 minutes (typical for optical imaging (IVIS) or ultrasound to more than an hour for MRI and PET/SPECT/CT. During the imaging procedures the animals will be unconscious under anesthesia. For long term anesthesia (MRI/PET/SPECT/CT) we will use a dedicated life monitoring system that will record respiration rate and control body temperature to minimize any negative impact on the condition of the animal by the duration of the anesthesia. For ultrasound imaging the hair on the skin will be shaved. Food fasting of animal (with access to water) for a maximum of 24 h can be part of the imaging procedure, such as for FDG. Moderate discomfort due to recovery from anesthesia.

Placement of s.c. estradiol pellet.
In case of hormone dependent tumors (e.g. mcf-7 cells), the s.c. placement of an estradiol pellet is required for tumor outgrowth. Placement is performed under a brief narcosis with isoflurane. Mild discomfort.

Resection of hormone producing tissues.
In order to mimic the situation in human patients that suffer from hormone-sensitive tumors, it may be required to perform a resection of the hormone-producing tissues (testes, ovaries). Surgery will be performed under general anesthesia and appropriate analgesia. Moderate discomfort.

In appendix 3 we describe the use of metastases models for interventions that are directed to metastases. As outlined in the project proposal form, part of these metastatic models will be derived from the primary transplantable tumor models described in this appendix. In this case resection of the primary tumor may be required for studying treatment of metastatic disease. In that case, we will surgically remove the primary tumor under general anesthesia and appropriate analgesia, before the humane endpoint has been reached. Next, the animal will be monitored for the outgrowth of secondary metastases in distant organs. Severe discomfort due to metastatic outgrowth is possible. The number of animals used for this purpose will be justified in appendix 3.

3: Treatments
Treatment may involve any kind of agent (e.g. chemicals, biologicals, cells, genetic vectors, radiopharmaceuticals, vaccines) and or combination of those and may also include radiotherapy, acoustic therapy, thermal therapy or photodynamic therapy as required per study. The safety/tolerability of such (combinations of) treatments will be established first under the appendix 4 (tolerability studies) before being used as routine procedure for the treatment of animals in intervention studies. Agents will be administered by the appropriate route duration and frequency as required. Examples are bolus injections (i.v./i.p./oral), continuous infusion in cannulated animals, minipumps (ALZET), slow release pellets, topical application by direct application or skin tattooing. Simple procedures, mild discomfort. Procedures requiring surgery (e.g. minipumps, cannulations) will be performed under general anesthesia and analgesia resulting in moderate discomfort. The selection of the agent(s), dose and route of application depends on target of the intervention and the details of the treatment of each study will be discussed with the IvD.

4: Euthanasia
At the end of the study the animal will be killed by an approved method (e.g. CO_2 asphyxiation, cervical dislocation, terminal anesthesia). Mild discomfort.

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

A typical intervention study will comprise several study arms (control group(s) and treatment groups). To demonstrate a 50% improvement in terms of tumor growth rate or survival between two groups (test vs controls) with the overall variability (relative standard deviation) being around 30, we will need a group size of 8 evaluable animals per group (power > 0.9 with α = 0.05, two sided). However, if multiple treatment groups are included we need to increase the group size because we need to adjust for multiple comparisons (the α of 0.05 will be divided by the number of test groups minus 1 (e.g. 5-arm study will take α as 0.05/3 = 0.013). Overall, the group size will need to be 10 evaluable animals when performing a 5-arm study. In order to obtain sufficient evaluable animals we will need more animals from start as will be outlined below.
Randomization/stratification will take place once the tumor has reached a predefined size in a predefined time window. Animals that do not develop a tumor, or develop a tumor outside the predefined time window (too rapid, too slow) will be taken off-study (censored). Whenever possible we will use models with high tumor take and consistent growth characteristics, but some of the PDX models may have a lower take rate. Overall, we expect that om average about 25% cannot be used for this reason.
Consequently, we will need a group size of 13 animals per study arm, thus 5 x 13 = 65 animals per study.

B. The animals

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

Species: Mus Musculus.
Origin: own breeding or commercial breeder
Gender: Both male and female mice will be used, although per series one gender will generally be selected. Obviously, for certain tumors only one gender can be used (e.g. mammary tumors require female mice).
Justification: Mice are considered the most appropriate and, therefore, most frequently used animal model in oncology because of their short generation time and the ease of genetic modifications. Like humans, they are mammals with similar organ structures, sharing many similarities in genetic composition. There is a wealth of information on -omics data and many advanced bio-molecular tools for genetic modification are available. Moreover, there is a wide range of available tumor models, cell lines, xenografts that are transplantable into (immune compromised) mice.
We will use the adult mice of the strain that is required for the particular tumor type. These mice can be syngeneic to the tumor model or immune-compromised (nude mice, NSG mice) for non-syngeneic / xenograft models. In special cases where we will study the impact of drug transporting proteins on systemic and/or tumor exposure (e.g. important when assessing the efficacy of therapeutics against brain tumors) we will use nude mice or FVB, FVB/129Ola mice lacking these drug transporters (constitutive knockouts).

Estimated numbers:
We expect to perform 75 intervention studies with primary metastatic tumor models per year, resulting in a total of 75 x 65 = 4875 mice per year (**24,375 in 5 years**). Note, only about 80% of these will be actually be included in the studies and will undergo the discomfort of the interventions and handlings. The remaining 20% will not be eligible for study (e.g. no tumor take, wrong tumor, too long tumor latency) and will be humanely killed before discomfort due to tumor growth will occur.

New transplantable models that have not previously been tested in our facility must be tested to establish the tumor take and growth consistency (model consistency check), before these can be used for intervention studies. For this purpose, we will use 20 mice per new transplantable model to establish the

tumor take. We expect to test 20 new transplantable models per year, thus requiring 400 mice per year (**2,000 in 5 years**).

We will also need to induce tumors in animals for studying the PK-PD relationships. These experiments and the justification for the number of animals are written in appendix 4 (Pharmacology).

C. Re-use

Will the animals be re-used?

☒ No, continue with question D.

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

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

☐ No

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

D. Replacement, reduction, refinement

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

Cell culture experiments and other evaluations of the proposed concepts for interventions are being done prior to the in vivo experiments. The results thereof are evaluated critically and only if these tests/concepts are considered sufficiently promising, the step towards in vivo testing will be taken.
Since cancer is a complex disease, it is necessary to study the treatment of the disease in vivo. Cell culture, organoids or computer models, are not sophisticated enough (yet) for this purpose as the interaction between the tumor and host environmental factors such as the stroma, oxygen supply, the immune system and metabolism is not accounted for. In general, the transplantable tumor models will be the first in vivo models used to assess the efficacy of the experimental therapy as a first screen. When these models fail to show efficacy, it will mean that the investigational therapy in its current design will not be continued.
The proposed number of evaluable animals per study arm (n=10) is based on our experience with this type of experiments and in line with generally accepted protocols in the literature. Further reduction of animal numbers per cohort will deteriorate the statistical power. State-of-the-art methods and equipment for tumor induction and follow up of tumor growth (imaging) will be used to minimize discomfort to the animals.

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

We realize that the procedures/handlings that will be conducted under this protocol will inevitably cause suffering of the animals in these studies. In order to minimize suffering, we will adhere to the national (Code of Practice) and internationally accepted rules (Code of Practice) of handling lab animals in oncology (see P. Workman et al. Guidelines for welfare and use of animals in cancer research. Br J Cancer 2010; 102: 1555-1577). Under these rules, the animals will be humanely killed when the humane endpoint is reached. Within the Institute, we have standard operation procedures (SOP) for animal handlings. Importantly, this also includes a SOP for analgesia that should reduce suffering from pain to a minimum. Next to that, we will use state-of-the-art imaging techniques that allow for non-invasive follow up of tumor growth, which is important in case of tumors growing inside internal organs, as this will help to identify animals at risk for developing symptoms. While under anesthesia for imaging, the animals are kept in a temperature controlled environment. For long term imaging (MRI, PET/SPECT/CT) the respiration will be checked to balance the depth of the anesthesia using Life-monitoring systems.

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. The proposed research does not relate to legally required research

Accommodation and care

F. Accommodation and care

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

☒ No

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

G. Location where the animals procedures are performed

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

☒ No > Continue with question H.

☐ Yes > Describe this establishment.

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

Classification of discomfort / humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

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

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

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

Within the NKI, we have developed a standard operation protocol that describes the most appropriate / suitable methods for anaesthesia and analgesia for each (surgical) procedure. This protocol has been developed by the animal welfare officer of the NKI and may be subject to change when new concepts or

ideas about optimal anaesthesia/analgesia evolve.

I. Other aspects compromising the welfare of the animals

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

Animals carrying tumors in internal organs may develop dysfunction of involved organs or other complications (e.g. obstruction of airway or gastro-intestinal tract) just like cancer patients. Treatments may cause toxic side effects.

Explain why these effects may emerge.

These effects are a consequence of tumor growth and treatments

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

In general the negative effects on the well-being of the animals by the tumor cannot be prevented. In order to minimize the burden of the tumor, the animals will be monitored at a frequency that is dictated by the model and timely killed when the humane endpoint as described below is met. The safety and tolerability of treatments included in the intervention studies will have been established under appendix 4 (tolerability). Nevertheless, unforeseen complications may occur. In such cases, we will try to find solutions that will minimize the impact of the unforeseen complications, for example by providing easy access to food (mush-feeding), taking into account the humane endpoints as listed below.

J. Humane endpoints

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

☐ No > Continue with question K.

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

We will adhere to the Code of Practise of lab animals used in oncology, in line with internationally agreed rules (see P. Workman et al. Guidelines for welfare and use of animals in cancer research. Br J Cancer 2010; 102: 1555-1577). In general the most important humane endpoints that apply are:

- A weight loss of more than 20% of the initial body weight, measured from the start of the treatment and in case of adult animals. In case of juvenile animals, tailored rules will apply.
- A tumor mass greater than 10% of the body weight, usually 2000 cubic mm in case of more superficial measurable lesions (by caliper) and/or skin ulceration/necrosis.
- Severe abnormal breathing.
- Severe abnormal behavior.

Indicate the likely incidence.

We expect mild discomfort in about 35%, moderate discomfort in 55% and severe discomfort in 10% of the animals.

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').

Since this protocol contains different procedures as outlined in subsection A, we have also provided the discomfort associated with each procedure in subsection A for clarity.

In general, the tumor model used will determine the severity of the discomfort as categorized below (see also Table 1)
-Animals that will carry superficial tumors (subcutaneous or mammary fatpad) that do not cause symptomatic metastasis within the lifetime of the study

animal or severe weight loss, but do reach the endpoint of maximum tumor size will generally experience mild discomfort. Animals with subcutaneous tumors that ulcerate can experience moderate discomfort.
-Animals with tumors growing in internal organs but who are sacrificed at a time before the humane endpoints are reached (e.g. on the basis of non-invasive imaging results or used for tissue sampling for pharmacologic studies in appendix 4) will undergo moderate discomfort during the handlings for tumor induction.
-Animals with tumors growing in internal organs and used for measuring survival as readout, thus reaching one of the predefined humane endpoints can undergo severe discomfort.
- Animals that do not develop tumors or develop tumors in time (about 25% of all animals) will be taken out of study before experiencing more than mild discomfort.
Overall the majority of animals used under this appendix will carry a primary ectopic tumor and will therefore experience mild or moderate discomfort in 35 and 55% of cases, respectively. Orthotopic tumor models will be used much less and thus only about 10% of the animals under this appendix is expected to experience severe discomfort.

Table 1.

1a. Primary ectopic tumor models

Tumor source	**Origin**	**Host**	**Location of grafting**	**Metastatic capacity**	**Discomfort[1]**	**Take rate / consistency[2]**	**Remarks**
Cell line[3]	Mouse	Syngeneic	subcutaneous	Low to none	Mild to moderate	High, >80%[4]	In general low discomfort, unless ulcerating tumor occur (=humane endpoint)
Cell line	Human	Immune deficient		Low to none		High, >80%	
Patient Derived Xenograft (PDX)	Human	Immune deficient		Low to none		Variable, >50%	

1b. Primary orthotopic tumor models

Tumor source	**Origin**	**Host**	**Location of grafting**	**Metastatic capacity**	**Discomfort[1]**	**Take rate / consistency**	**Remarks**
Cell line[3]	Mouse	Syngeneic	Depends on origin of the tumor	Moderate/high	Mild to severe, depending on location	High, >80%	In general, it will be the primary tumor site that determines discomfort and not the metastases
Cell line	Human	Immune deficient		Moderate/high		High, >80%	
Patient Derived Xenograft (PDX)	Human	Immune deficient		Possible		Variable, 50%	

Induction of the tumor are expected to give the following level of discomfort:
Injection of tumor cells through the skin (subcutaneous tumors) under a brief narcosis with isoflurane: mild discomfort.
Surgical implantation of tumor cells into the animal under general anesthesia: moderate discomfort.

Any of the techniques / interventions below is not expected to alter / enhance the total level of discomfort:

[1]When grown until humane endpoint is reached

[2] Consistency: The tumor needs to reach a predefined size within a predefined time window for inclusion into a study cohort.

[3] Cell line can either involve injection of a cell suspension or implantation of tissue fragments

[4] Expected average percentage of eligible animals for intervention studies

-Simple well tolerated interventions (drug treatments) will cause discomfort classified as mild.
-More intensive interventions / treatments and those requiring sedation (image guide radiotherapy) may cause discomfort classified as moderate.
-Simple but frequent handlings like weighing and caliper measurements will cause discomfort classified as mild.
-More intensive handlings, especially those requiring sedation (placement of infusion pumps or non-invasive imaging) will cause discomfort classified as moderate.
-Castration or ovariectomy will be result in discomfort graded as moderate.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

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

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

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

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

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

☒ Yes

Appendix
Description animal procedures

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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: 02 Type of animal procedure: Intervention protocol for genetically engineered mice with spontaneous or somatically induced tumors

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

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

This appendix describes the use of advanced mouse models as a surrogate for cancer patients to validate targets that can be used as anti-cancer therapy, for cancer prevention and/or can be used to circumvent host-related or tumor-related factors that may cause therapy resistance. For this purpose, tumor-bearing mice will be used to assess the antitumor effects of pharmacological, radio-therapeutic and/or genetic interventions. This appendix describes the use of genetically engineered mouse models (GEMM's) of specific genotypes that develop spontaneous tumors from germline or GEMM's that develop tumors after

somatic induction. The selection of a specific spontaneous germline model or a somatically inducible model is based on the type and/or genetic abnormalities in the human tumors that we want to mimic. GEMMS's are more complex and an intervention study using a GEMM will require more animals, because of less consistent tumor formation and possible occurrence of concurrent other tumors (e.g. skin). For this reason we will use GEMM only after the particular intervention has shown to be efficacious in transplantable tumor models (confirmatory studies) or as first model when other (in vitro) data suggest that the use of such complex models is required from start. We have a set of GEMM models available (see Table 1 in the project proposal form), but expect that this list will grow as several groups are working on the development of additional GEMM models (under current DEC projects or future CCD project licenses).

The development of these tumors and their response to treatment will be monitored by appropriate techniques and tumors will be treated. The readout can be based on size (visual or imaging), survival (humane endpoints) and/or biological effect (based on analysis following the resection/collection of relevant tissue material). The aim of these experiments is to develop better treatments that may be applied / translated to the clinic.

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

1: Tumor induction

1a: Spontaneous formation of tumors from specific germline mutations.
Tumors will develop spontaneously (without further intervention) during the lifetime of the animal due to the genotype. Mice need to be of specific genotype, i.e. contain one or more genetically modified genes and in general carry a transgene that drives tissue-specific expression of Cre-recombinase that together cause malignant transformation of organ specific host cells. An example is our mouse model for Brca-deficient mammary tumors, where the mice carry homozygous conditionally (loxP flanked) alleles for Bcra1 and P53 and a mammary gland specific WAPCre or KCre transgene.

1b:Somatic induction of tumors
Induction of tumors in GEMM animals occurs from local or systemic injection/application of agents/vectors that cause transformation of normal cells. Such agents/vectors can be viral vectors, Crispr/Cas, chemicals or specific agents such as tamoxifen for CreERT models. The technique for induction depends on the model that is required to mimic the specific cancer. For example we will use intra-tracheal instillation of CMV-Cre adenoviral particles for modelling non-small cell lung cancer in GEMM mice carrying a LoxP conditional $Kras^{v12}$ mutant transgene and homozygous loxP conditional P53 alleles. All applications will be performed under appropriate anesthesia and analgesia as required per procedure. The handlings for somatic inductions usually involve surgical techniques with anesthesia and analgesia, classified as mild or moderate discomfort.

As a result of the induction procedures (somatic or spontaneous), tumors will develop, but time of onset and speed of growth will vary per model. Animals will be monitored using appropriate techniques and at a frequency that is appropriate for the model. The discomfort caused by the tumor will depend on the model. In general, mild to moderate for more superficial tumors, such as skin and mammary tumors that do not cause symptomatic metastases. The discomfort caused by tumors growing inside internal organs can be severe when grown to a size that the humane endpoints are reached (e.g. loss of body weight >20% of the initial weight, abnormal breathing, abnormal posture, etc.).

2: Tumor development and progression follow-up

Palpation and/or caliper measurement of the tumor can be used to measure tumor growth in case of superficial tumors. To this end, the mouse will be fixed by hand and the tumor palpated. Tumors larger than 3 mm will be measured by calipers. Mild discomfort.

In cases of follow up of tumor volume when tumors are not superficial and/or to obtain biological information from the tumor (growing superficial and inside

internal organ sites), we will need to use appropriate imaging techniques (IVIS, SPECT, MRI, PET, CT and ultrasound). Examples are to monitor distribution of a radiolabeled antibody or to measure apoptosis by ^{99m}TC-Annexin. As part of the imaging procedure, the injection of a radioactive tracer (for PET and SPECT) or contrasting agent (MRI, CT, ultrasound) may be used. If not yet available, these imaging procedures will be developed and validated under a separate CCD project license. In some cases, tumor cells have been transduced with soluble luciferase and blood sampling (25 ul from the tail) can then be used to monitor tumor growth. Mild discomfort.

Duration and frequency varies between 5-10 minutes (typical for optical imaging (IVIS) or ultrasound to more than an hour for MRI and PET/SPECT/CT. During the imaging procedures the animals will be unconscious under anesthesia. For long term anesthesia (MRI/PET/SPECT/CT) we will use a dedicated life monitoring system that will record respiration rate and control body temperature to minimize any negative impact on the condition of the animal by the duration of the anesthesia. For ultrasound imaging the hair on the skin will be removed. Food fasting of animal (with access to water) for a maximum of 24 h can be part of the imaging procedure, such as for FDG. Moderate discomfort due to recovery from anesthesia

In appendix 3 we describe the use of metastases models for interventions that are directed to metastases. As outlined in the project proposal form, part of these metastatic models will be derived from GEMM models described in this appendix. In this case resection of the primary tumor may be required for studying treatment of metastatic disease. In that case, we will surgically remove the primary tumor under general anesthesia and appropriate analgesia, before the humane endpoint has been reached. Next, the animal will be monitored for the outgrowth of secondary metastases in distant organs. Severe discomfort due to metastatic outgrowth is possible. The number of animals used for this purpose will be justified in appendix 3.

3: Treatments
Treatment may involve any kind of agent (e.g. chemicals, biologicals, cells, genetic vectors, radiopharmaceuticals, vaccines) and or combination of those and may also include radiotherapy, acoustic therapy, thermal therapy or photodynamic therapy as required per study. The safety/tolerability of such (combinations of) treatments will be established first under the appendix 4 (tolerability studies) before being used as routine procedure for the treatment of animals in intervention studies. Agents will be administered by the appropriate route, duration and frequency as required. Examples are bolus injections (i.v./i.p./oral), continuous infusion in cannulated animals, minipumps (ALZET), slow release pellets, topical application by direct application or skin tattooing. Simple procedures, mild discomfort. Procedures requiring surgery (e.g. minipumps, cannulations) will be performed under general anesthesia and analgesia resulting in moderate discomfort. The selection of the agent(s), dose and route of application depends on target of the intervention and the details of the treatment of each study will be discussed with the IvD.

4: Euthanasia
At the end of the study the animal will be killed by an approved method (e.g. CO_2 asphyxiation, cervical dislocation, terminal anesthesia). Mild discomfort.

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

A typical intervention study will comprise several study arms (control group(s) and treatment groups). To demonstrate a 50% improvement in terms of tumor growth rate or survival between two groups (test vs controls) with the overall variability (relative standard deviation) being around 30, we will need a group size of 8 evaluable animals per group (power > 0.9 with $\alpha = 0.05$, two sided). However, if multiple treatment groups are included we need to increase the group size because we need to adjust for multiple comparisons (the α of 0.05 will be divided by the number of test groups minus 1 (e.g. 5-arm study will take

α as 0.05/4 = 0.013). Overall, the group size will need to be 10 evaluable animals when performing a 5-arm study. In order to obtain sufficient evaluable animals we will need more animals from start as will be outlined below.
Randomization/stratification will take place once the tumor has reached a predefined size in a predefined time window. Animals that do not develop a tumor, or develop a tumor outside the predefined time window (too rapid, too slow) will be taken off-study (censored). We expect that about 10% cannot be used for this reason. GEMMS may also develop other tumors. For example K14cre transgenic breast cancer models develop skin cancers in about 30% of cases and these mice need to be removed prior to randomization.
Consequently we will need a group size of 14 animals per study arm, thus 5 x 14 = 70 animals per study.

B. The animals

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

Species: Mus Musculus
Origin: own breeding or commercial breeder
Gender: Both male and female mice will be used whenever possible. Obviously, for certain tumors only one gender can be used (e.g. mammary tumors require female mice).
Justification: Mice are the most appropriate and, therefore, most frequently used animal model in oncology because of their short generation time and the ease of genetic modifications. Like humans, they are mammals with similar organ structures, sharing many similarities in genetic composition. There is a wealth of information on -omics data and many advanced bio-molecular tools for genetic modification are available. The tumor models in this appendix are based on genetically engineered models that are only available in mice.
We will use GEMM mice with a genotype that is required for the particular tumor type. These mice will be generated under active breeding programs at the institute or may be obtained via other institutes or purchased from commercial breeders. In general, tumor models that are used for intervention studies have a short to intermediate latency time, but for tumor models developing from germline it may take more than 8-10 months.
Estimated numbers:
We expect to perform 25 intervention studies with GEMM tumor models per year, resulting in a total of 25 x 70 = 1750 mice per year (**8,750 in 5 years**). Note, only about 70% of these will be actually be included in the studies and will undergo the discomfort of the interventions and handlings. The remaining 30% will not be eligible for study (e.g. no tumor take, wrong tumor, too long tumor latency) and will be humanely killed before discomfort due to tumor growth will occur.

We will also need to induce tumors in GEMM animals for studying the PK-PD relationships. These experiments and the justification for the number of animals are written in appendix 5 (Pharmacological studies).

C. Re-use

Will the animals be re-used?

☒ No, continue with question D.

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

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

☐ No

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

D. Replacement, reduction, refinement

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

Cell culture experiments and other evaluations of the proposed concepts for interventions will be done prior to the in vivo experiments. The results thereof are evaluated critically and only if these tests/concepts are considered sufficiently promising, the step towards in vivo testing will be taken.
Since cancer is a complex disease, it is necessary to study the treatment of the disease in vivo. Cell culture, organoids or computer models, are not sophisticated enough (yet) for this purpose as the interaction between the tumor and host environmental factors such as the stroma, oxygen supply, the immune system and metabolism is not accounted for. In general, these more complex GEMMS's will be used when in vivo studies with simpler (transplantable) tumor models have demonstrated the potential of the intervention, but now need to be tested in a more stringent model. The proposed number of evaluable animals per study arm (n=10) is based on our experience with this type of experiments. Further reduction of animal numbers per cohort will deteriorate the statistical power. State-of-the-art methods and equipment for tumor induction and follow up of tumor growth (imaging) will be used to minimize discomfort to the animals.

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

We realize that the procedures/handlings that will be conducted under this protocol will inevitably cause suffering of the animals. In order to minimize suffering, we will adhere to the national (Code of Practice) and internationally accepted rules of handling lab animals in oncology (see P. Workman et al. Guidelines for welfare and use of animals in cancer research. Br J Cancer 2010; 102: 1555-1577). Under these rules, the animals will be humanely killed when the humane endpoint is reached. Within the Institute, we have standard operation procedures (SOP) for animal handlings. Importantly, this also includes a SOP for analgesia that should reduce suffering from pain to a minimum. Next to that, we will use state-of-the-art imaging techniques that allow for non-invasive follow up of tumor growth, which is important in case of tumors growing inside internal organs, as this will help to identify animals at risk for developing symptoms. While under anesthesia for imaging, the animals are kept in a temperature controlled environment. For long term imaging (MRI, PET/SPECT/CT) the respiration will be checked to balance the depth of the anesthesia using Life-monitoring systems.

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. The proposed research does not relate to legally required research

Accommodation and care

F. Accommodation and care

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

☒ No

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

G. Location where the animals procedures are performed

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

☒ No > Continue with question H.

☐ Yes > Describe this establishment.

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

Classification of discomfort / humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

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

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

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

Within the NKI, we have developed a standard operation protocol that describes the most appropriate / suitable methods for anaesthesia and analgesia for each (surgical) procedure. This protocol has been developed by the animal welfare officer of the NKI and may be subjected to change when new concept or ideas about optimal anaesthesia/analgesia evolve.

I. Other aspects compromising the welfare of the animals

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

Animals carrying tumors in internal organs may develop dysfunction of involved organs or other complications (e.g. obstruction of airways or gastro-intestinal tract) just like cancer patients. Treatments may cause side effects.

Explain why these effects may emerge.

These effect are a consequence of tumor growth and treatments

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

In general the negative effects on the well-being of the animals by the tumor cannot be prevented. In order to minimize the burden of the tumor, the animals will be monitored at a frequency that is dictated by the model and timely killed when the humane endpoint as described below is met. The safety and tolerability of treatments included in the intervention studies will have been established under appendix 4 (tolerability). Nevertheless, unforeseen

complications may occur. In such cases, we will try to find solutions that will minimize the impact of the unforeseen complications, for example by providing easy access to food (mush-feeding), taking into account the humane endpoints as listed below.

J. Humane endpoints

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

☐ No > Continue with question K.

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

We will adhere to the Code of Practise of lab animals used in oncology, in line with internationally agreed rules (see P. Workman et al. Guidelines for welfare and use of animals in cancer research. Br J Cancer 2010; 102: 1555-1577). In general the most important humane endpoints that apply are:

- A weight loss of more than 20% of the initial body weight, measured from the start of the treatment and in case of adult animals. In case of juvenile animals, tailored rules will apply.
- A tumor mass greater than 10% of the body weight, usually 2000 cubic mm in case of more superficial measurable lesions (by caliper) and/or skin ulceration/necrosis.
- Severe abnormal breathing.
- Severe abnormal behavior.

Indicate the likely incidence.

We expect mild discomfort in about 50%, moderate discomfort in 25% and severe discomfort in 25% of the animals.

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').

Since this protocol contains different procedures as outlined in subsection A, we have also provided the discomfort associated with each procedure in subsection A for clarity.

In general, the tumor model used will determine the severity of the discomfort as categorized below:
-Animals with melanoma or breast cancer that form superficially growing tumors and do not cause symptomatic metastasis within the lifetime of the study animal or other major symptoms, but do reach the endpoint of maximum tumor size will generally experience mild/moderate discomfort.
-Animals with tumors growing in internal organs but who are sacrificed at a time before the humane endpoints are reached (e.g. on the basis of non-invasive imaging results or used for tissue sampling for pharmacologic studies in appendix 4) will undergo moderate discomfort during the handlings for tumor induction.
-Animals with tumors growing in internal organs and used for measuring survival as readout, thus reaching one of the predefined humane endpoints may undergo severe discomfort.
- Animals that do not develop tumors in time or the wrong tumors (about 30% of all animals) will be taken out of study before experiencing more than mild discomfort.

Table 1							
2. Spontaneous or inducible Transgenic models (GEMMs)							
Location	**Tumor Type**	**Host**	**Induction**	**Metastatic capacity**	**Discomfort[1]**	**Take rate / consistency**	**Remarks**
Brain	Glioblastoma	Inducible	lentiviral-Cre	none	Severe	High, 70-90%	Locally invasive, neurologic symptoms
Breast	Ductal carcinoma (DC) based on Brca loss	Spontaneous inducible	K14Cre/WAPCre/ lenti-or adenoviral-Cre	Low	Mild/Moderate	High, 60-90%	About 30% of KCre will develop skin tumors and cannot be used
Breast	Invasive Lobular carcinoma (ILC) based on Ecad loss	Spontaneous Inducible	K14Cre/WAPCre/ lenti-or adenoviral-Cre	Moderate/high	Mild/Moderate	High, >60-80%	About 30% of KCre will develop skin tumors and cannot be used.
Breast	ILC models based on PI3K/Akt mutations	Spontaneous Inducible	K14Cre/WAPCre/ lenti-or adenoviral-Cre	Low	Mild/Moderate	High >80%	Locally invasive. About 30% of KCre will develop skin tumors and cannot be used.
Breast	Her2+ BC	Spontaneous	MMTV-NeuT	Low	Mild/Moderate	High >80%	
Lung	NSCLC	Inducible	adenoviral-Cre	Low	Moderate/Severe	High, >70-90%	Breathing distress at end stage
	SCLC	Inducible	adenoviral-Cre	Moderate/high	Moderate/Severe	High, >70-90%	Ditto, Long lag time (8 months)
	Mesothelioma	Inducible	adenoviral-Cre	Moderate	Moderate/Severe	High, >70-90%	Breathing distress at end stage
Skin	Melanoma	Inducible	Cre-ERT/Tamoxifen	Low/moderate	Mild/Moderate	Medium, 40-60%	50% develop other skin tumors

Breast cancer models are most widely used for our research and (as shown in Table 1) these usually cause mild or moderate discomfort. For this reason, we expect that animals under this appendix (GEM models) will experience mild, moderate and severe discomfort in 50%, 25% and 25% of cases.

Additional discomfort due to procedures:
Induction of the tumor formation is expected to give the following level of discomfort:
Injection/application of inducing agent on, intra or percutaneous (melanoma, mammary fatpad tumors and mesothelioma) under a brief narcosis with isoflurane: mild discomfort.
Surgical application of inducing agent into the animal under general anesthesia (intra-tracheal intubation, intracranial injection): moderate discomfort.

Any of the techniques / interventions below is not expected to alter / enhance the total level of discomfort:
-Simple well tolerated interventions (drug treatments) will cause discomfort classified as mild.
-More intensive interventions / treatments and those requiring sedation (image guide radiotherapy) may cause discomfort classified as moderate.
-Simple but frequent handlings like weighing and caliper measurements will cause discomfort classified as mild.
-More intensive handlings, especially those requiring sedation (placement of infusion pumps or non-invasive imaging) will cause discomfort classified as moderate.
-Castration or ovariectomy will be result in discomfort graded as moderate.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

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

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

In general, the condition of the animals at the end of the experiment will require that the animal is humanely killed.

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

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

☒ Yes

Appendix
Description animal procedures

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

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	30100
1.2	Provide the name of the licenced establishment.	Stichting Het Nederlands Kanker Instituut – Antoni van Leeuwenhoek ziekenhuis
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 03 Type of animal procedure: Intervention protocol for recipient mice with metastatic tumors

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

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

This appendix describes the use of metastatic tumor models in mice as a surrogate for cancer patients to validate targets that can be used as anti-cancer therapy and/or can be used to circumvent host-related or tumor-related factors that may cause therapy resistance. To this end, tumor-bearing mice will be used to assess the antitumor effects of pharmacological, radio-therapeutic and/or genetic interventions. This appendix describes the use of mice in which metastatic lesions are the subject of the intervention. Metastatic lesions can be induced by injection of tumor cells into the blood circulation after which cells

will form tumors at distant sites (e.g. tail vein injection causing lung metastases), hereafter called experimental metastases models. Metastatic lesions can also be formed spontaneously from the transplantable or the GEMM tumor models (as described in appendices 1 and 2, respectively). In order to use these so called spontaneous metastases models, it will often be necessary to perform a resection of the primary tumor before the primary tumor will grow to a size that will require sacrifice of the animal (humane endpoints). The spontaneous metastases models will mimic the clinical situation more closely, where recurrence following resection of the primary tumor is the leading cause of death.
The main difference between spontaneous metastatic models from transplantable models and GEMMs is that the former occur from more end-stage like tumors, whereas the latter also includes the earlier steps in tumorigenesis and metastasis. GEMM models, however, require more animals, because of less consistent tumor take and possible occurrence of concurrent other tumors. For this reason, we will use GEMM models for metastases when the earlier events must be taken into account for the particular intervention. In most other cases, we will prefer the transplantable models for metastases.

Due to technical constraints, surgical resection of a primary tumor in mice will only be feasible in more accessible (superficial) tumors, such as skin and mammary tumors. Besides technical feasibility, the selection of the specific tumor type will be based on the type and/or genetic abnormalities of the tumors in patients that we want to mimic. The growth and behavior of these tumors will be monitored by appropriate techniques and animals will be treated by the investigational treatment. The readout can be based on tumor progression (visual or imaging), survival (humane endpoints) and/or biological effect (based on analysis following the resection/collection of relevant tissue material). The aim of these experiments is to develop better treatments and/or obtain results that may be applied / translated to the clinic.

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

1. Metastases induction can be achieved by three routes:

a: Metastases from transplantable (primary) tumor models:
Note: the animal procedures for transplantable models have been described in detail in appendix 1

As a result of the transplantation procedures (orthotopic or ectopic), tumors will develop, but time of onset and speed of growth will vary per model. For these studies we will use models with a high tumor take rate and high frequency of forming metastases in a predefined time window. In particular some of our syngeneic tumor cell lines and PDX models of breast cancer implanted in the mammary fatpad are suited for this purpose.
Growth of the primary tumor will be monitored using appropriate techniques and at a frequency that is appropriate for the model. Once the primary tumor has reached a predefined size (about 750 cubic mm for a superficial lesion) within the predefined time window, the superficial tumor will undergo resection under general anesthesia and appropriate analgesia. Local recurrences may occur in 20% of animals and will be a reason to censor the animal.

b. Metastases from genetically engineered mouse models (GEMMs) of cancer:
Note: the animal procedures for GEMMs has been described in detail in appendix 2

In GEMMs, tumors will develop spontaneously or following somatic induction, but time of onset and speed of growth will vary per model as outlined in appendix 2. For these studies we will use GEMMs with a high tumor take rate and high frequency of forming metastases. In particular ductal carcinoma (DCs) and invasive lobular carcinomas (ILCs) of the breast are suited for this purpose. They are growing more confined and are thus easily amenable to complete surgical resection. Nevertheless, local recurrences may occur in about 20% of cases.
Tumor growth will be monitored using appropriate techniques and at a frequency that is appropriate for the model. When amenable to surgical resection (superficially growing primary tumors), the tumor will be resected under general anesthesia and appropriate analgesia when it has reached a predefined size (usually about 750 cubic mm) in a predefined time window.

c. Metastases occurring from injection of tumor cells in the blood circulation (experimental metastases models):
Metastases can form when tumor cell suspensions are injected into the blood stream and subsequently home to distant tissues. These are generally more robust models in terms of tumor take rate and growth consistency, because they do not depend on stochastic events like initial tumor cell dissemination from the primary tumor and survival of tumor cells in the central circulation. However, this latter is also a downside of the model, since it cannot be used to study the effects that interventions may exert on tumor cell dissemination from the primary tumor. Therefore, these experimental metastases models cannot always replace the spontaneous metastases models.
We have the following experimental metastases routes/models:
-Intravenous injection into the tail vein resulting in lung metastases. Requires only a single i.v. injection (mild discomfort). Occasionally local tumors in the tail may form due to extravasation of tumor cells during the injection. Usually these local tumors remain very small, but if not may become a reason to censor the animal.
-Intra-splenic injection resulting in liver metastases. Involves surgery under general anesthesia and adequate analgesia. Two minutes after the injection, the spleen will be removed (95% of cells will have reached the liver) to prevent outgrowth of a primary tumor in the spleen. Cauterization is used to avoid bleeding. Bleeding may occur (5-10% of cases) and requires immediate killing of the animal. Moderate discomfort.
-Intra-carotid arterial injection resulting in brain metastases. Involves microsurgery under general anesthesia and adequate analgesia. Success rate in skilled hands about 90%. Moderate discomfort.
-Intra-cardiac injection resulting in generalized metastases. Is performed under general anesthesia (isoflurane) and analgesia. Requires exposure of the chest wall / ribs through a small skin incision in order to locate the correct spot for intra-cardiac injection in between the ribs through the chest wall. Immediate respiratory problems due to internal bleeding can occur but is not very frequent (<5% of cases).

2: Tumor development and progression follow-up

In order perform intervention studies in metastatic tumors we will need to use appropriate imaging techniques (BLI, SPECT, MRI, PET, CT and ultrasound). For this purpose we will work mainly with tumors that express luciferase in order to allow bioluminescence imaging (BLI) for easy follow up. However, interventions in immune-proficient mice may be hindered by cells expression ectopic proteins, such as luciferase, which is especially problematic when immunology based interventions are being studied. Alternatively, we can use survival as primary study outcome or we can take out tissues at predefined time points to quantify or characterize the metastatic lesions by ex vivo analysis (e.g. FACS).
Overall, due to the location, metastatic tumors have a high probably of causing moderate or severe discomfort.

Imaging: Duration and frequency varies between 5-10 minutes (typical for optical imaging (IVIS) or ultrasound to more than an hour for MRI and PET/SPECT/CT. During the imaging procedures the animals will be unconscious under anesthesia. For long term anesthesia (MRI/PET/SPECT/CT) we will use a dedicated life monitoring system that will record respiration rate and control body temperature to minimize any negative impact on the condition of the animal by the duration of the anesthesia. For ultrasound imaging the hair on the skin will be shaved. Food fasting of animal (with access to water) for a maximum of 24 h can be part of the imaging procedure, such as for FDG. Moderate discomfort due to recovery from anesthesia.

3: Treatments
Treatment may involve any kind of agent (e.g. chemicals, biologicals, cells, genetic vectors, radiopharmaceuticals, vaccines) and or combination of those and may also include radiotherapy, acoustic therapy, thermal therapy or photodynamic therapy as required per study. The safety/tolerability of such (combinations of) treatments will be established first under the appendix 4 (tolerability studies) before being used as routine procedure for the treatment of

animals in intervention studies. Agents will be administered by the appropriate route duration and frequency as required. Examples are bolus injections (i.v./i.p./oral), continuous infusion in cannulated animals, minipumps (ALZET), slow release pellets, topical application by direct application or skin tattooing. Simple procedures, mild discomfort. Procedures requiring surgery (e.g. minipumps, cannulations) will be performed under general anesthesia and analgesia resulting in moderate discomfort. The selection of the agent(s), dose and route of application depends on target of the intervention and the details of the treatment of each study will be discussed with the IvD.

4: Euthanasia
At the end of the study the animal will be killed by an approved method (e.g. CO_2 asphyxiation, cervical dislocation, terminal anesthesia). Mild discomfort.

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

A typical intervention study will comprise several study arms (control group(s) and treatment groups). To demonstrate a 50% improvement in terms of tumor growth rate or survival between two groups (test vs controls) with the overall variability (relative standard deviation) being around 30, we will need a group size of 8 evaluable animals per group (power > 0.9 with α = 0.05, two sided). However, if multiple treatment groups are included we need to increase the group size because we need to adjust for multiple comparisons (the α of 0.05 will be divided by the number of test groups minus 1 (e.g. 5-arm study will take α as 0.05/4 = 0.013). Overall, the group size will need to be 10 evaluable animals when performing a 5-arm study. In order to obtain sufficient evaluable animals we will need more animals from start as will be outlined below.

In case of a metastases model from a transplanted tumor we need 70 mice per study. Randomization/stratification will take place once the primary tumor has reached a predefined size in a predefined time window. Animals that do not develop a tumor, or develop a tumor outside the predefined time window (too rapid, too slow) will be taken off-study (censored). We expect that about 10% cannot be used for this reason.
We will also need to censor animals that develop a local recurrence following tumor resection. We expect that about 20% of the animals cannot be used for this reason. The group size will further depend on the fraction of animals in the untreated control group that remain disease free without treatment (which is than due to a lack of metastasis formation). For this reason, we will only use tumor models with a known high metastases formation rate (>90%). In this case we will need a group size of 14 animals per study arm, thus 5 x 14 = 70 animals per study.

In case of a metastases model from a GEMM we need 80 animals per study. Randomization/stratification will take place once the primary tumor has reached a predefined size in a predefined time window. Animals that do not develop a tumor, or develop a tumor outside the predefined time window (too rapid, too slow) will be taken off-study (censored). We will generally use models with a high and consistent tumor take, but expect that about 10% cannot be used for this reason. The breast cancer GEMM that is mostly used as metastases model (ILC from conditional Ecad;P53 mice) also develop other tumors in about 30% of cases (mainly skin tumors) and these mice need to be removed prior to randomization.
We will also need to censor animals that develop a local recurrence following tumor resection. We expect that about 20% of the animals cannot be used for this reason. ILCs from Ecad;P53 mice have a 100% metastatic rate and for this model we will need a group size of 16 animals per study arm, thus 5 x 16 = 80 animals per study.

In case of a metastases model following the injection of a tumor cell suspension into the blood stream we need 60 animals per study. we will use only models with a robust metastatic tumor take (>90%). Due to technical issues (e.g. bleeding events) we will need an additional 10% extra mice. Thus for the experimental metastases models we will need a group size of 12 per study arm, thus 5 x 12 = 60 animals.

B. The animals

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

Species: Mus Musculus.
Origin: own breeding or commercial breeder
Gender: Both male and female mice will be used, although per series one gender will generally be selected. Obviously, for certain tumors only one gender can be used (e.g. mammary tumors require female mice).
Justification: Mice are considered the most appropriate and, therefore, most widely used animal model in oncology because of their short generation time and the ease of genetic modifications. Like humans, they are mammals with similar organ structures, sharing many similarities in genetic composition. There is a wealth of information on -omics data and many advanced bio-molecular tools for genetic modification are available. Moreover, there is a wide range of available tumor models, cell lines, xenografts that are transplantable into (immune compromised) mice.
We will use the adult mice of the strain that is required for the particular tumor type. These mice can be syngeneic to the tumor model or immune-compromised (nude mice, NSG mice) for non-syngeneic / xenograft models. In special cases where we will study the impact of drug transporting proteins on systemic and/or tumor exposure (e.g. important when assessing the efficacy of therapeutics against brain tumors) we will use nude mice or FVB, FVB/129Ola mice lacking these drug transporters (constitutive knockouts)
Estimated numbers:
We expect to perform 6 intervention studies with primary metastatic tumor models per year, resulting in a total of 6 x 70 = 420 mice per year
We expect to perform 4 intervention studies with GEMM metastatic tumor models per year, resulting in a total of 4 x 80 = 320 mice per year
We expect to perform 4 intervention studies with tumor cell suspensions per year, resulting in a total of 4 x 60 = 240 mice per year
Total = 420 + 320 + 240 = 980 per year (**4,900 in 5 years**).

Model consistency check:
New metastatic models that have not previously been tested in our facility must be tested to establish the tumor take and growth consistency, before these can be used for intervention studies. For this purpose, we will use 25 mice per new transplantable model to establish the tumor take. We expect to test 2 new transplantable models per year, thus requiring 50 mice per year (**250 in 5 year**)

Total number of animals for this appendix: **5,150 in 5 year**

C. Re-use

Will the animals be re-used?

☒ No, continue with question D.

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

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

☐ No

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

D. Replacement, reduction, refinement

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

Cell culture experiments and other evaluations of the proposed concepts for interventions are being done prior to the in vivo experiments. The results thereof are evaluated critically and only if these tests/concepts are considered sufficiently promising and/or required to address a specific scientific question, the step towards in vivo testing will be taken.
Since cancer is a complex disease, it is necessary to study the treatment of the disease in vivo. Cell culture, organoids or computer models, are not sophisticated enough (yet) for this purpose as the interaction between the tumor and host environmental factors such as the stroma, oxygen supply, the immune system and metabolism is not accounted for. The proposed number of evaluable animals per study arm (n=10) are based on our experience with this type of experiments and in line with generally accepted protocols in the literature. Further reduction of animal numbers per cohort will deteriorate the statistical power. State-of-the-art methods and equipment for tumor induction and follow up of tumor growth (imaging) will be used where possible to minimize discomfort to the animals.

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

We realize that the procedures/handlings that will be conducted under this protocol will inevitably cause suffering of the animals in these studies. In order to minimize suffering, we will adhere to the national (Code of Practice) and internationally accepted rules of handling lab animals in oncology (see P. Workman et al. Guidelines for welfare and use of animals in cancer research. Br J Cancer 2010; 102: 1555-1577). Under these rules, the animals will be humanely killed when the humane endpoint is reached. Within the Institute, we have standard operation procedures (SOP) for animal handlings. Importantly, this also includes a SOP for analgesia that should reduce suffering from pain to a minimum. Next to that, we will use state-of-the-art imaging techniques that allow for non-invasive follow up of tumor growth, which is important in case of tumors growing inside internal organs, as this will help to identify animals at risk for developing symptoms. While under anesthesia for imaging, the animals are kept in a temperature controlled environment. For long term imaging (MRI, PET/SPECT/CT) the respiration will be checked to balance the depth of the anesthesia using Life-monitoring systems.

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. The proposed research does not relate to legally required research

Accommodation and care

F. Accommodation and care

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

☒ No

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

G. Location where the animals procedures are performed

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

☒ No > Continue with question H.

☐ Yes > Describe this establishment.

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

Classification of discomfort / humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

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

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

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

Within the NKI, we have developed a standard operation protocol that describes the most appropriate / suitable methods for anaesthesia and analgesia for each (surgical) procedure. This protocol has been developed by the animal welfare officer of the NKI and may be subject to change as new concepts or ideas about optimal anaesthesia/analgesia evolve.

I. Other aspects compromising the welfare of the animals

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

Animals carrying tumors in internal organs may develop dysfunction of involved organs or other complications (e.g. obstruction of airway or gastro-intestinal tract) just like cancer patients. Treatments may cause toxic side effects.

Explain why these effects may emerge.

These effects are a consequence of tumor growth and treatments

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

In general the negative effects on the well-being of the animals by the tumor cannot be prevented. In order to minimize the burden of the tumor, the animals will be monitored at a frequency that is dictated by the model and timely killed when the humane endpoint as described below is met. The safety and tolerability of treatments included in the intervention studies will have been established under appendix 4 (tolerability). Nevertheless, unforeseen complications may occur. In such cases, we will try to find solutions that will minimize the impact of the unforeseen complications, for example by providing easy access to food (mush-feeding), taking into account the humane endpoints as listed below.

J. Humane endpoints

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

☐ No > Continue with question K.

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

We will adhere to the Code of Practise of lab animals used in oncology, in line with internationally agreed rules (see P. Workman et al. Guidelines for welfare and use of animals in cancer research. Br J Cancer 2010; 102: 1555-1577). In general the most important humane endpoints that apply are:

- A weight loss of more than 20% of the initial body weight, measured from the start of the treatment and in case of adult animals. In case of juvenile animals, tailored rules will apply.
- A tumor mass greater than 10% of the body weight, usually 2000 cubic mm in case of more superficial measurable lesions (by caliper) and/or skin ulceration/necrosis.
- Breathing distress.
- Severe abnormal behavior.

Indicate the likely incidence.

We expect mild discomfort in about 25%, moderate discomfort in 25% and severe discomfort in 50% of the animals.

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').

Since this protocol contains different procedures as outlined in subsection A, we have also provided the discomfort associated with each procedure in subsection A for clarity.

In general, the tumor model will determine the severity of the discomfort as categorized below:
-Animals with metastatic tumors growing inside internal organs, but sacrificed at a time that the humane endpoints are not yet reached (e.g. on the basis of non-invasive imaging results and/or used for tissue harvesting will undergo discomfort during the handlings for tumor induction as outlined above.
-Animals with metastatic tumors growing inside internal organs and used for measuring survival as readout, thus reaching one of the predefined humane endpoints can undergo severe discomfort.
- Animals that do not develop tumors (within the time window) will be taken out of study before experiencing more than mild discomfort.
- Resection of the superficial primary tumor will be done in animals that carry a primary tumor of a size resulting in not more than mild discomfort. However, resection of the primary tumor may result in moderate discomfort.

Table 1 Metastasis models

a. Any of the primary tumor models described in appendix 1, where the primary tumor will be resected before tumor burden reaches the humane endpoint

Location, tumor type, host and induction			**Metastatic capacity**	**Discomfort[1]**	**Take rate / consistency**	**Remarks**
Model dependent			Sufficient	Mild/moderate	Variable	
b. Cell suspensions injected directly into the blood stream and forming lesions at (a) distant site(s)						
Metastatic site	**Site of injection**	**Handling**				
Lung	Tail vein	Easy		Severe	High, >70-90%	
Liver	Spleen (+ subsequent splenectomy)	Moderate		Severe	High, >70-90%	
Brain	carotid artery	Complex		Severe	High, >70-90%	
General	Cardiac (left ventricle)	Moderate		Severe	Moderate, >50%	

As described in appendix 1, the induction of the transplantable tumor are expected to give the following level of discomfort:
Injection of tumor cells through the skin (subcutaneous tumors) under a brief narcosis with isoflurane: mild discomfort.
Surgical implantation of tumor cells into the animal under general anesthesia: moderate discomfort.

Metastatic tumor when grown to the size that the humane endpoint is reached will cause severe discomfort. We expect that animals under this appendix may suffer from severe discomfort in 50% of cases. In those cases where animals do not develop metastases or where the animal will be sacrificed based on imaging results, the discomfort level will be less (mild or moderate).

Any of the techniques / interventions below is not expected to enhance the overall level of discomfort:
-Simple well tolerated interventions (drug treatments): mild discomfort.
-More intensive interventions / treatments and those requiring sedation (image guide radiotherapy): moderate discomfort.
-Simple but frequent handlings like weighing and caliper measurements: mild discomfort.
-More intensive handlings, especially those requiring sedation (placement of infusion pumps or non-invasive imaging: moderate discomfort.
-Castration or ovariectomy: moderate discomfort.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

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

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

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

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

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

☒ Yes

Appendix
Description animal procedures

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

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	30100
1.2	Provide the name of the licenced establishment.	Stichting Het Nederlands Kanker Instituut – Antoni van Leeuwenhoek ziekenhuis
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 04 Type of animal procedure: Testing the tolerability of investigational treatments

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 purpose of these procedures is to determine the tolerability of the proposed investigational treatment before it will be used in subsequent efficacy studies.

In the case that we plan to conduct an intervention study with a new small molecule drug compound that will be used in mice for the first time, we will first

conduct a pilot pharmacokinetic study (as described in appendix 5, Pharmacological studies), before conducting the maximum tolerated dose (MTD) of this agent, in order to confirm that the test agent has appropriate drug-like properties (sufficient metabolic stability and sufficient systemic exposure). If the systemic exposure of the experimental agent is considered inadequate this will be a no-go decision point for further studies with this agent.

In most cases, the investigational treatment will involve agents that have previously been tested but in our planned experiments they will be used in combination with other agents (e.g. small molecule drugs, biologicals, cells, genetic vectors, radiopharmaceuticals, vaccines) and/or may be combined with other treatment modalities (radiotherapy, acoustic therapy, thermal therapy or photodynamic therapy) as required per study.

The safety/tolerability of the investigational treatments will be established using non-tumor bearing animals, before it will be used as intervention in tumor bearing animals. We will use mice of the same strain as planned for the efficacy study. Cohorts of animals will be exposed to a range of exposure levels and closely monitored for signs of discomfort. These studies will be mainly observational (scoring well-being of the animal, body weight), but may include sampling of blood to assess physiological parameters such as routine hematology (Hb, WBC) and clinical chemistry parameters (e.g. liver enzymes, creatinine, electrolytes), T-cell response, cytokines and other appropriate readouts. Animals will be humanely killed when the humane endpoints are reached.

As mentioned above, these tolerability studies will be done in non-tumor bearing animals. However, if there are indications that the presence of a tumor is necessary for the correct interpretation, we will confirm the safety also in an additional cohort of tumor-bearing animals, using the information in the non-tumor bearing animals in order to minimize the number of animals in which we will need to induce a tumor. The induction of tumors is described in the appendices 1 - 3.

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

Treatment may involve any kind of agent (e.g. small molecules, biologicals, cells, genetic vectors, radiopharmaceuticals, vaccines) and or combinations thereof and may also include radiotherapy, acoustic therapy, thermal therapy or photodynamic therapy as required per study. The safety/tolerability of such (combinations of) treatments will be established under this appendix before it will be used for intervention studies in tumor bearing animals.

In most cases, these tolerability studies will be used to determine maximum safe dose levels of the involved (combinations of) agents, i.e. the maximum tolerated dose (MTD). Agents will be administered by the appropriate route, duration and frequency as required during the intervention study. Examples are bolus injections (i.v./i.p./oral), continuous infusion of cannulated animals, osmotic minipumps (ALZET), slow release pellets, topical application by direct application or skin tattooing. The drugs can be given in combination with other treatment modalities that may affect the health status of the animal or the effect of the agent, such as for example radiotherapy.

Several schedules can be followed to find the appropriate dosing regimen. The choice of the strategy will depend strongly on the amount of information about the safety of the agent(s) that is already available. Guidelines of dose-finding strategies are given below (A-D). Ultimately, however, fine-tuning of the methodology may be required and the exact design will be assessed together by the investigator, the intervention team (pharmacologist) and the IVD.

A. If a presumed safe starting dose is available from the literature or otherwise (e.g. data-on-file from collaborators), we will use this dose and select 2 additional (most appropriate) dose levels based on educated estimation in order to test the safety in our animals under the conditions in our own animal facility. For this type of work we will need 15 animals (5 per dose level and 3 dose levels). The animals will receive the treatment by the same route / frequency / duration as planned in the (proposed) study design of the intervention study. Animals will be observed daily and body weight will be recorded. The humane endpoint is defined by loss of body weight by more than 15% within 48 h or more than 20% from the initial body weight or other

symptoms causing more than moderate discomfort. Acute transient effects after drug administration, such as "freezing", inactivity, anxiety are considered tolerable when they resolve within 15-30 min after drug administration and are causing not more than moderate discomfort. The MTD is the highest dose level where none of the animals needs to be sacrificed because of reaching the humane endpoints. A re-test using 10 animals (at slightly adapted dose levels) may be required to confirm the MTD. Per compound 25 mice per MTD determination in total.

B. If there is no information available on a safe starting dose (first-in-mouse study) of the treatment. We will obtain this information by first performing a pilot using cohorts of 2 animals and up to 4 different dose levels (8 animals) to cover a broad dose range in which we expect to find the MTD. Based on the outcome of this pilot, we will determine the MTD as described under 1A. Per compound 25 (1A) + 8 (1B) = 33 mice per MTD determination in total.

C. If we plan to use a new combination of agents, of which we do have information of each of the single agent modalities, we will select up to 6 different dose combinations to assess the safety in cohorts of 5 mice per combination each (30 mice). A re-test using 15 mice (at slightly adapted dose levels) may be required to confirm the MTD. (45 mice in total)

D. If we plan to use a combination of treatments and there is no information available on the MTD of one or more (mean = 2 unknowns) of the single modality treatments, we will first assess the MTD of the single modalities as described under 1B (2x8=16 mice). Next we will continue with step 1C (45 mice). In total: 45 (C) + 16 (D) = 61 mice per MTD determination.

Discomfort may be severe for animals treated above the MTD, but we expect that this will only be a small subset (<20%) of the animals. We will minimize suffering of those animals by careful monitoring and by the timely sacrifice in case of more than moderate toxicity.

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

Before starting the intervention studies it is very important to have a good assessment of the MTD that allows safe dosing of the animals. Taking too few animals increases the chance of unexpected excessive toxicities during the intervention studies. If this would happen, it will impoverish or annul the predictive value of the intervention study that includes tumor bearing animals.
The ultimate read-out of the MTD study is discrete (e.g. does or does not reach the humane endpoint), but continuous data will be collected (e.g. body weight data) that will help to make a good assessment whether a dose is close to the MTD or not. We will need 5 animals per dose level to assess its safety and will reconfirm this in a subsequent experiment whenever necessary to be confident about the outcome before continuing with the intervention studies.

.

B. The animals

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

The MTD and pilot pharmacokinetic studies will be established in non-tumor bearing animals of the same strain and gender as the tumor bearing animals that will be used in the intervention studies. These mice can be syngeneic to the tumor model or immune-compromised (nude mice, NSG mice) for non-syngeneic / xenograft models. In case efficacy studies are planned in transgenic mice, we will try to make use of the littermates of the tumor bearing animals that do not have the exactly correct genotype for tumor induction. For example, in case of studies with WAP-Cre;Brca1;P53 mice that will develop breast cancer, we

can use WAP-Cre negative female littermates (25% of the offspring) in order to closely match the genetic background of the study animals for intervention. All mice originate from active breeding protocols within the NKI or may be purchased from commercial breeders.

1. We expect to perform MTD studies for 25 treatments per year. The average number of mice needed per MTD determination (through any of the schedules 1A-1D) will be 40 mice per MTD study. Accordingly, we expect to use 1000 mice per year for this part of the work (**5,000 in 5 years**)

C. Re-use

Will the animals be re-used?

☒ No, continue with question D.

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

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

☐ No

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

D. Replacement, reduction, refinement

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

This appendix describes the procedures that are necessary to determine a safe dose before subsequent efficacy studies and there are no accurate in vitro or in silico models available as alternative for in vivo testing. It is important to know the safety of the treatment before conducting efficacy studies in tumor bearing animals, otherwise there is a chance that the efficacy study (which includes more animals that have been induced with a tumor) would need to be terminated prematurely, when excessive toxicity occurs. Thus being more conservative in animal numbers during the MTD studies may be paid back in fewer prematurely terminated efficacy studies involving tumor induced animals.

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

The suffering of the animals in the MTD studies will be minimized by careful monitoring of the animals in study and timely sacrifice when the condition of the animal deteriorates. Weight loss will be a guiding parameter, but all other signs of discomfort will be taken into account. In general, the dose regimens should be well tolerated when implementing them in further in efficacy studies. Consequently, animals that receive a toxic dose during the MTD studies can be rapidly killed, because these doses are not applicable for subsequent efficacy studies.

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. The proposed research does not relate to legally required research

Accommodation and care

F. Accommodation and care

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

☒ No

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

G. Location where the animals procedures are performed

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

☒ No > Continue with question H.

☐ Yes > Describe this establishment.

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

Classification of discomfort / humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

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

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

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

Where necessary, adequate analgesia and/or general anesthesia will be applied

I. Other aspects compromising the welfare of the animals

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

Next to the immediate effects of the treatments during the MTD studies as outlined above, we do not expect other adverse effects

Explain why these effects may emerge.

The treatment are causing the effects. They are inevitable to occur in the subgroup of the animals that will receive a dose that will be above the MTD.

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

We will minimize the severity by close observation of the animals in study and by timely sacrifice of animals receiving doses above the MTD

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.

We will adhere to the Code of Practise of lab animals used in oncology, in line with internationally agreed rules (see P. Workman et al. Guidelines for welfare and use of animals in cancer research. Br J Cancer 2010; 102: 1555-1577). In general the most important humane endpoint that applies to these MTD studies is weight loss of more than 15% in 48 hours or 20% of the initial body weight, measured from the start of the treatment and in case of adult animals.

Other humane endpoints are not expected to be met during these studies (e.g. a tumor mass greater than 10% of the body weight, usually 2000 cubic mm in case of more superficial measurable lesions (by caliper) and/or skin ulceration/necrosis, severe abnormal breathing (indicative of tumor lesions in the lungs).

Indicate the likely incidence.

We expect that during the MTD studies, overall about 20% of the animals may receive a dose levels that will require sacrifice due to meeting the humane endpoints.

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').

Animals used for the MTD studies may experience up to severe toxicity due to the treatments. We expect that this may involve about 20% of the animals. We expect to minimize this discomfort by timely sacrifice of the animals when we observe that the dose level in a particular cohort is above the MTD.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

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

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

Due to the experiment, the condition of a part of the animals will require that the animal is humanely killed. Other animals cannot be re-used in other studies and will also be killed.

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

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

☒ Yes

Appendix
Description animal procedures

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

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	30100
1.2	Provide the name of the licenced establishment.	Stichting Het Nederlands Kanker Instituut – Antoni van Leeuwenhoek ziekenhuis
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 05 Type of animal procedure: Pharmacokinetic-Pharmacodynamics (PK-PD) studies

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 experiments described in this appendix have the following objectives:

1. Determine the pharmacokinetics of agents used for intervention studies in order to assess the most optimal route and schedule for therapy, to verify that the systemic exposure will be sufficient to expect an effect on the tumor, but not so high that the levels are clinically irrelevant (*i.e.* cannot be reached in cancer patients) and/or to identify potential drug-drug interactions when working with more than one agent.

2. Study the relationship between systemic drug exposure, tumor distribution, biological response and antitumor response (pharmacokinetic/ pharmacodynamics relationships.

Ad. 1. When the investigational treatment involves the administration of an agent (drug, biological), we will need to establish the pharmacokinetic behavior of this entity. For this purpose, the agent(s) will be administered by the appropriate dose and route followed by sampling of blood and/or tissues at specified time points to determine the levels of the agent(s) and/or putative metabolites. These studies will be conducted when the existing information (from own previous studies of from literature data) is not sufficient or lacking. We will use non-tumor bearing animals of the same strain that will be used in the actual intervention studies.
In the case that we plan to conduct an intervention study with a new investigational compound that will be used in mice for the first time, we will first conduct a pilot pharmacokinetic study, even before conducting the MTD studies, to confirm that the test agent has appropriate drug-like properties (sufficient metabolic stability and sufficient systemic exposure). Failure to demonstrate appropriate drug-like properties will be a no-go decision point. Usually n=5 animals dosed i.v. or i.p. and sampled via the tail vein (as described under ad 1.1 in the next section) will be sufficient.
In case that the drug target needs to be exposed to the agent for a certain period of time, we need to confirm adequate systemic drug exposure during that period. This information is important, because it will determine the dosing scheme (dose level and time laps in between repeated drug administrations). Usually sampling over a period of 24 h period is required.
In case that oral administration is required (e.g. because of clinical relevance), we will need to assess the plasma concentration *vs* time curves both after i.v. dosing and after oral dosing in order to calculate the bioavailability ($F = AUC_{oral}:AUC_{i.v.}$).
In case that we want to confirm absence or presence of a drug-drug interaction between two agents, we will need to assess the plasma concentration *vs* time curves of each agent when given alone and in combination.
For the treatment of brain tumors, where drug penetration is hindered by drug transporters in the blood-brain barrier, we will assess the drug exposure in the brain of animals that are proficient or deficient (knockout) for the transporters.

Ad. 2. Essential information about the success or failure of the investigational intervention studies (as assessed in appendices 1-3) will be obtained by studying the relationship between systemic exposure, tumor exposure, biological response (e.g. target inhibition) and antitumor response. For this purpose, cohorts of animals carrying the tumor model that is used in the intervention studies will be exposed to the investigational treatment(s) and relevant biological materials (e.g. blood and/or other body fluids and tumor and other tissues) will be collected at preset time points after start of therapy for subsequent analyses. The treatment may involve the use of multiple dose levels (levels of exposure) in order to establish the relationships between systemic and tumor drug levels and biological response (*e.g.* level of target inhibition, number of infiltrative T cells)

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

Ad 1.1 In order to establish the pharmacokinetics of the investigational compounds, animals will receive the test agent(s) by the administration route as planned for the intervention studies.

When just a small blood volume (up to 50 ul) is sufficient to determine the concentration of the test compound, blood will be sampled from the tip of the tail at several time intervals, up to a maximum of 6 times or less if we need larger blood samples than 50 ul (maximum total blood volume taken: 300 ul). The last sampling point will involve a terminal bleeding by cardiac puncture under general anesthesia (isoflurane) and tissues will be harvested at that point.
The number of animals that we need will depend on the time span after drug administration over which we need to determine drug levels.

When using the method of repeated sampling we will need:
-5 animals for drug level monitoring over a time period of 0 and 4 h: sampled at t = 5, 15, 30 min, 1, 2 and 4 h

-10 animals for drug level monitoring over a time period of 0 and 24 h: n= 5 sampled at t = 5, 15, 30 min, 1, 2 and 4 h, and n=5 at t = 15 min, 1, 4, 7, 12 and 24 h.
The timing is indicative and may vary depending on the most appropriate timing for the particular agent(s). After the last sampling point, the animal will be used for terminal bleeding under isoflurane anesthesia and killed by cervical dislocation for harvesting of tissues.

In cases where we cannot use blood sampling from the tail (large volume required or tail vein sampling not compatible with drug administration via the tail) we will perform terminal bleeding by cardiac puncture, using 5 animals per time point. To cover the complete plasma concentration time curve we will need up to 6 time points: generally 15 min, 1 h, 4 h, 7 h, 12 h and 24 h (5 x 6 = 30 animals).

In case we are comparing between different strains or genotypes (e.g. in case of brain tumor studies where we must assess the role of drug transporting proteins expressed at the blood/brain barrier), the number of animals required will be doubled when comparing 2 strains (Abcb1a/b;Abcg2 KO vs WT mice).

Discomfort due to the handlings as listed above will be mild.

Ad 2.1 For these studies, we will use mice carrying the same tumor model as will be used in the intervention study. Procedures for the induction of these tumors have been outlined in appendices 1-3.
The pharmacokinetic/ pharmacodynamics (PK/PD) studies will be carried out on two time points after drug administration, usually around the peak plasma level (C_{max}) and at the plasma trough level (C_{trough}). The number of animals per time point will depend on the number of parameters that need to be determined and the size of the tumor. In general, we require 5 replicates per cohort for continuous (quantitative) data (e.g. drug levels, FACS analyses) and 3 replicates for discrete (semi-quantitative) data (e.g. immunocytochemistry, western blot). We will compare one or more treatment cohort(s) with a cohort of untreated control animals.

Relatively large subcutaneous or mammary fatpad tumors are usually big enough to obtain sufficient material from one tumor to assess all parameters (e.g. drug levels, FACS analyses, DNA/RNA isolation, western blotting immunocytochemistry, histology). Thus of 5 control group animals + 10 of treatment group animals (5 mice at 2 time points) = 15 mice will be needed.

Tumors growing inside internal organs (e.g. brain tumors) or subcutaneous tumors that need to be used at a time point when they are still very small may provide sufficient material for just one parameter at a time. We expect that we will assess two PD parameters per treatment (e.g. western blot and histology), Per parameter: n=3 for control + n=6 (3 x 2) for the test groups = 9 mice. Two parameters: 2 x 9 = 18 mice. For the PK studies we will need an additional n=10 mice per treatment group to assess the drug levels in the tumor. Total 28 mice.

Discomfort: Discomfort due to the handling as listed above will be mild. The potential discomfort due to the tumor induction and proliferation has been addressed in appendices 1-3.

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

Pharmacokinetic studies:
Based on long term experience with this type of experiments we expect to need 5 animals per time point in order to account for the inter-animal variability in drug handling for an accurate assessment of the systemic exposure.

Pharmacokinetics / Pharmacodynamics relationship in tumor bearing animals:
These studies do involve comparisons between (untreated) controls and treatment groups. In case of continuous read-out, we want to discriminate a 50% change with a power of 0.9, α=0.05 and an inter-animal variability of 25%. In this case we will need 5 animals per group. For semi-quantitative data, such as comparison by western blot, n=3 per group will generally suffice.

B. The animals

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

The pharmacokinetics will be established in non-tumor bearing animals of the same strain and gender and age as the tumor bearing animals that will be used in the intervention studies. These mice can be syngeneic to the tumor model or immune-compromised (nude mice, NSG mice) for non-syngeneic / xenograft models. In special cases where we will study the impact of drug transporting proteins on systemic and/or tumor exposure (e.g. important when assessing the efficacy of therapeutics against brain tumors), we will make use of nude mice lacking these drug transporters. In case of the GEMM tumor models, we will make use of the littermates of the tumor bearing animals that do not have the exactly correct genotype for tumor induction. For example, in case of studies with WAP-Cre;Brca1;P53 mice that will develop breast cancer, we can use WAP-Cre negative female littermates (25% of the offspring) in order to closely match the genetic background of the study animals for intervention. All mice originate from active breeding protocols within the NKI or may be purchased from commercial breeders.

1. We expect that we need to assess the plasma concentration time profiles of 50 compounds/combinations of compounds/routes of administration per year. The average number of mice used per study will be 20. Accordingly, we expect to use 1000 mice per year for this part of the work.
2. We expect to conduct PK-PD studies with 50 treatments in tumor bearing animals per year. The average number of mice used per PK-PD study will be 20. Accordingly, we expect to use 1000 mice per year for this part of the work.

In total we will use 2000 animals per year under this appendix (**10,000 in 5 years**).

C. Re-use

Will the animals be re-used?

☒ No, continue with question D.

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

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

☐ No

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

D. Replacement, reduction, refinement

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

Cell culture experiments and other evaluations of the proposed concepts for interventions will be done prior to the in vivo experiments. The results thereof

are evaluated critically and only if these tests/concepts are considered sufficiently promising and/or required to address a specific scientific question, the step towards in vivo testing will be taken.
Since cancer is a complex disease, it is necessary to study PK and PK/PD relationships of the treatment of the disease in vivo. In vitro cell culture, organoids or computer models, are not sophisticated enough (yet) for this purpose as the interaction between absorption, distribution, metabolism and excretion (ADME) of the drug and and host environmental factors on the tumor, such as the stroma, oxygen supply, the immune system and metabolism is not accounted for. Mice are considered the most appropriate and, therefore, most frequently used animal model in oncology.

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

Suffering of the animals in the PK and PK/PD studies is generally limited to mild discomfort only. The dose levels that are used are well tolerated, the duration of the studies is relatively short and the handlings (drug administration, tail vein blood sampling) are fairly mild or conducted under terminal anesthesia (cardiac puncture).

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. The proposed research does not relate to legally required research

Accommodation and care

F. Accommodation and care

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

☒ No

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

G. Location where the animals procedures are performed

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

☒ No > Continue with question H.

☐ Yes > Describe this establishment.

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

Classification of discomfort / humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

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

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

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

Where necessary (terminal bleeding), adequate analgesia and/or general anesthesia will be applied

I. Other aspects compromising the welfare of the animals

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

We do not expect more than mild adverse effects

Explain why these effects may emerge.

The drugs and their formulations may cause some mild effects, but for this type of study we will use dose levels that are well tolerated

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

We will minimize the severity by close observation of the animals in study and by timely sacrifice of animals that display unexpected side effects leading to more than mild discomfort

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.

We will adhere to the Code of Practise of lab animals used in oncology, in line with internationally agreed rules (see P. Workman et al. Guidelines for welfare and use of animals in cancer research. Br J Cancer 2010; 102: 1555-1577). Humane endpoints are not expected to be met during these short-term PK and PK/PD studies.

Indicate the likely incidence.

We expect that only in very rare cases animals may undergo more than mild discomfort.

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').

Animals used in the PK and PK/PD studies will generally undergo only mild discomfort due to the handlings of the drug administrations and samplings. For

the PK/PD studies we will use tumor bearing mice and the induction of tumor (as described in appendices 1 - 3) may result into moderate discomfort.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

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

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

Animals used for PK and PK/PD studies will be killed as part of the study for harvesting blood and tissues.

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

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

☒ Yes

Dierexperimentencommissie NKI
Plesmanlaan 121
1066 CX AMSTERDAM
[redacted]

NETHERLANDS CANCER INSTITUTE
ANTONI VAN LEEUWENHOEK

A. Algemene gegevens over de procedure

1. Aanvraagnummer
2. Titel van het project: Pre-clinical intervention studies in mice for prevention and treatment of cancer.
3. Titel van de NTS: Preklinische interventiestudies in muizen voor kankerpreventie of -behandeling.
4. Type aanvraag:
   - ☐ nieuwe aanvraag projectvergunning
5. Contactgegevens DEC:
   - Naam DEC: DEC NKI
   - Telefoonnummer contactpersoon: [redacted], bereikbaar op[redacted]
   - Mailadres contactpersoon: [redacted]
6. Adviestraject:
   - ☐ ontvangen door DEC: 31-12-2015
   - ☐ aanvraag compleet: 31-12-2015
   - ☐ in vergadering besproken: 13-01-2016
   - ☐ anderszins behandeld: n.v.t.
   - ☐ termijnonderbreking(en) van 20-01-2016 tot 23-01-2016
   - ☐ besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen n.v.t.
   - ☐ aanpassing aanvraag: n.v.t.
   - ☐ advies aan CCD: 27-01-2016
7. Eventueel horen van aanvrager
   - Datum n.v.t.
   - Plaats
   - Aantal aanwezige DEC-leden
   - Aanwezige (namens) aanvrager
   - Strekking van de vraag / vragen
   - Strekking van het (de) antwoord(en)
   - Het horen van de aanvrager heeft geleid tot aanpassing van de aanvraag
8. Correspondentie met de aanvrager
   - Datum: 20-01-2016
   - strekking van de vragen:
   - Algemeen: kunt u in de aanvraag duidelijk maken of de huidige, lopende experimenten met interventies al dan niet zullen worden ondergebracht onder deze vergunning?
   - Algemeen: de DEC heeft geadviseerd om op een aantal punten de tekst van de aanvraag te verhelderen en één lijn te hanteren bij het vermelden van allerlei zaken in de verschillende bijlagen.
   - Datum: 23-01-2016
   - strekking van de antwoorden:

- Aanvrager heeft onder punt 3.4.3 van het project formulier duidelijk gemaakt dat de lopende interventiestudies allemaal een korte duur hebben en dat het onderbrengen van die lopende studies onder de nog te verlenen vergunning niet zinvol zou zijn. Na verlening van de vergunning worden geen nieuwe interventiestudies (als bedoeld in de huidige aanvraag) gestart onder nog lopende projecten van voor 18 december 2014.
- Aanvrager heeft een aantal tekstuele wijzigingen in de aanvraag aangebracht die tot de gewenste duidelijkheid hebben geleid.

9. Eventuele adviezen door experts (niet lid van de DEC): n.v.t.
   - Aard expertise
   - Deskundigheid expert
   - Datum verzoek
   - Strekking van het verzoek
   - Datum expert advies
   - Expert advies

B. Beoordeling (adviesvraag en behandeling)

1. Het project is vergunningplichtig.
2. De aanvraag betreft een nieuwe aanvraag.
3. De DEC is competent om hierover te adviseren.

C. Beoordeling (inhoud):

1. Het project is:
   - uit wetenschappelijk oogpunt verantwoord.

2. De in de aanvraag aangekruiste doelcategorieën, fundamenteel en translationeel onderzoek, zijn in overeenstemming met de hoofddoelstelling.

3. De DEC is overtuigd van het belang van de doelstelling van het onderzoek. *In vivo* preklinisch onderzoek in muizen is onmisbaar om de resultaten van fundamenteel kankeronderzoek en nieuwe inzichten op basis van observaties bij patiënten, te kunnen vertalen naar nieuwe en verbeterde interventies in de kliniek. Het preklinisch testen van (combinatie)therapieën en preventieve interventies geeft informatie over zaken als werkzaamheid, veiligheid, farmacokinetiek en -dynamiek. Zonder informatie over de vraag of de tumor door de interventie ook *in vivo* daadwerkelijk wordt voorkomen, afgeremd of teruggedrongen, over de vraag wat een veilige dosering is en over de vraag hoe lang een stof in het lichaam blijft en of een stof na toediening de tumor wel bereikt, is verder onderzoek in mensen niet goed mogelijk en ook ethisch niet aanvaardbaar. De commissie classificeert het belang daarom als essentieel.

4. Deze aanvraag heeft geen betrekking op een traditioneel project, waarvan de doelstelling behaald is als de vraagstelling beantwoord is. Dit project bestaat uit een groot aantal kleine, relatief kortdurende studies die er ieder voor zich op gericht zijn om de waarde van een bepaalde (combinatie)therapie te onderzoeken in een daarvoor geschikt ziektemodel in de muis. Vaak zal op basis van de eerste bevindingen in muizen en in patiënten, maar ook op basis van nieuwe wetenschappelijk inzichten een therapie worden aangepast, om zo, op basis van voortschrijdend inzicht, tot steeds betere therapieën te komen. De doelstelling van het project is dus om gedurende de looptijd van het project te werken aan het vernieuwen en verbeteren van kankertherapieën, teneinde deze in de kliniek te kunnen introduceren.

Om te kunnen spreken van een toetsbare eenheid is het naar de mening van de commissie nodig dat er een helder zicht is op de doelstelling, het belang van die doelstelling en de haalbaarheid daarvan, op de gevolgen voor de gezondheid en het welzijn van de dieren en op de mate waarin beschikbare alternatieven ook daadwerkelijk toegepast worden. Dit project wordt gevormd door een groot aantal kleine, relatief kortdurende interventiestudies, die tekens dezelfde aanpak kennen. Ze zijn op een vergelijkbare wijze opgebouwd uit een aantal stappen die worden genoemd in antwoord op vraag 3.4.2 van het “projectgedeelte” van de aanvraag. In de aanvraag wordt duidelijk beschreven op basis van welke criteria wordt besloten een (nieuwe) “behandelingsvorm” te testen in een interventiestudie in dieren. Aan de dierexperimenten gaat een fase van in vitro experimenten en/of literatuurstudie vooraf. Daarna wordt stap voor stap bepaald wat de maximaal getolereerde dosis is, wat het meeste geschikte tumormodel is, of de behandelingsvorm effectief is en of de resultaten verder onderzoek met deze behandelingsvorm rechtvaardigen. Voor elke stap is helder geschetst aan welke voorwaarden moet worden voldaan om de volgende stap te kunnen zetten (go/no go beslismomenten). Ook is helder aangegeven welke keuzes binnen de verschillende stappen kunnen worden gemaakt en op basis van welke criteria dat gebeurt (bijvoorbeeld de keuze voor een bepaald type tumormodel). De verschillende typen tumormodellen en de gevolgen daarvan voor het welzijn van de dieren worden uitvoerig beschreven, waardoor per te gebruiken dier duidelijk wordt wat de gevolgen zijn voor de gezondheid, het welzijn en de integriteit van het dier. De drie V's zijn nadrukkelijk onderdeel van de criteria op basis waarvan voor een bepaald type tumormodel gekozen wordt. Bij elk experiment wordt actief gezocht naar het model dat het minste ongerief veroorzaakt en de minste dieren vergt door te letten op een voor het dier zo gunstige mogelijke locatie en eigenschappen van de tumor, hoge tumortake en weinig uitval.

Nieuwe inzichten in de moleculaire pathologie van kanker laten zien dat er grote overeenkomsten kunnen zijn tussen tumortypes die zijn ontstaan in verschillende organen en dat die gemeenschappelijke kenmerken ook relevant zijn voor de behandeling van deze tumoren. Het antwoord op de vraag wat de meeste effectieve behandeling vormt voor een bepaalde tumor wordt in hoge mate bepaald door de moleculaire eigenschappen van de tumor en de interactie van de tumor met zijn directe omgeving en het immuunsysteem. De kracht van een bundeling van alle tumorsoorten in één project ligt erin dat op deze manier optimaal onderzoek kan worden verricht naar de beste behandelmethode op basis van de onderliggende moleculair pathologische afwijkingen. Overeenkomsten en verschillen tussen tumortypes van diverse origine kunnen zo op de meest optimale manier worden onderzocht. Om die reden ligt het niet voor de hand om een project in te dienen dat zich richt op tumoren op één bepaalde anatomische locatie. Evenmin ligt het bij deze aanvraag voor de hand om deze te beperken tot één bepaalde behandelingsmodaliteit. De interventiestudies zullen zich veelal juist richten op het testen van een nieuwe behandelingsvorm in combinatie met een aantal andere behandelingsmodaliteiten.

De commissie ziet de aanvraag als een toetsbare eenheid en acht de gekozen strategie en experimentele aanpak adequaat voor het behalen van de doelstelling. Het NKI beschikt over de expertise, de muismodellen en de faciliteiten en apparatuur (Mouse Cancer Clinic) om dit onderzoek te kunnen doen. Het NKI heeft van NWO een “roadmap” subsidie gekregen voor het opzetten van deze onderzoeksfaciliteit, die qua uitrusting sterk lijkt op de kliniek waar humane patiënten worden behandeld.

5. Er is geen sprake van bijzonderheden op het gebied van categorieën van dieren, omstandigheden of behandeling van de dieren.

6. Het ongerief wordt in hoofdzaak bepaald door het feit dat tumoren worden geïnduceerd, die vervolgens subcutaan of orthotoop in de muizen groeien en in een klein aantal gevallen ook kunnen metastaseren. De tumoren worden behandeld en het effect van de behandeling wordt gevolgd. De behandeling zelf en het volgen van het effect van de behandeling met bijvoorbeeld beeldvormende technieken, kunnen

eveneens ongerief veroorzaken. Naar verwachting zal bij 47% van de dieren licht ongerief optreden en bij ca. 38% van de dieren matig ongerief. In ca. 15% van de dieren kan de groei van de tumor of de behandeling tot ernstig ongerief leiden. Zodra dat ontdekt wordt, is dat aanleiding om de dieren uit de proef te nemen en op humane wijze te doden. Het ongerief als gevolg van deze handelingen is realistisch ingeschat en geclassificeerd en in overeenstemming met wat in het kankeronderzoek gebruikelijk is. De commissie ziet geen reden daarvan af te wijken.

7. Er zijn geen methoden die de voorgestelde dierproeven geheel of gedeeltelijk zouden kunnen **vervangen.** De experimenten in de dieren zijn een noodzakelijke tussenstap op weg naar implementatie van nieuwe en verbeterde therapieën in de kliniek. Het testen van de effectiviteit van kandidaat kankertherapieën gebeurt voor een groot deel eerst in cellijnen en in andere *in vitro* modelsystemen. Kanker is echter een complexe ziekte die in zijn volle omvang alleen goed nagebootst kan worden in een compleet en levend organisme dat qua fysiologie in voldoende mate op de mens lijkt. In laatste instantie is altijd een “proof of principle” in een levend organisme nodig, onder andere vanwege de interactie tussen de tumor en zijn omgeving, de rol van het immuunsysteem, de bloedvoorziening naar de tumor en het feit dat de effectiviteit van toegediende middelen in belangrijke mate ook bepaald wordt door de vraag of deze middelen de tumor voldoende kunnen bereiken. De doelstelling van het project kan dus niet gerealiseerd worden zonder proefdieren of door gebruik van minder complexe diersoorten.

8. In het project wordt optimaal tegemoet gekomen aan de vereiste van de **vermindering** van dierproeven. Er wordt steeds goed op gelet dat de studies worden uitgevoerd met tumormodellen met een zo hoog mogelijke hoge tumortake en zo weinig mogelijk uitval. De aantallen dieren in de afzonderlijke studies zijn statistisch goed onderbouwd. Daarbij wordt gebruik gemaakt van historische data en van realistische aannames over te verwachten verschillen en spreiding. Het maximale aantal van 55.275 dieren in het gehele project in de komende vijf jaar is gebaseerd op de onderzoeksomvang in het recente verleden en op de beschikbare onderzoekscapaciteit. De commissie realiseert zich dat dit een zeer groot aantal dieren is. Dit is een gevolg van het feit dat het fundamentele onderzoek in snel tempo nieuwe inzichten genereert met betrekking tot de mechanismen die ten grondslag liggen aan het ontstaan en verloopt van kanker. Verwacht mag worden dat er een voortdurend toenemende vraag zal zijn naar translationeel onderzoek waarin de therapieën die op basis van die nieuwe inzichten zijn ontworpen, worden onderzocht. De commissie denkt dat de beschikbare onderzoekscapaciteit de beperkende factor zal zijn en dat dit feit op zichzelf al een reden voor de onderzoekers vormt om selectief te werk te gaan en alleen veelbelovende therapieën in muizen te testen.

9. Het project is in overeenstemming met de vereiste van de **verfijning** van dierproeven. Bij de keuze van tumormodellen wordt steeds met zorg gezocht naar mogelijkheden om de onderzoeksvraag te beantwoorden met het minst belastende model. Zo worden orthotope en metastaserende tumoren nadrukkelijk alleen gebruikt wanneer dat noodzakelijk is voor de beantwoording van de onderzoeksvraag. Bij het onderzoek wordt de “Code of Practice” voor het kankeronderzoek gevolgd. De gegevens die beschikbaar komen door het volgen van de ontwikkeling van de tumor, bijvoorbeeld door middel van beeldvorming, worden ook gebruikt om zo nauwkeurig mogelijk een humaan eindpunt te bepalen. Getrainde medewerkers houden toezicht op het welzijn van de dieren en zorgen voor de juiste toepassing van de humane eindpunten zoals die worden beschreven in de “Code of Practice”. Bij alle chirurgische handelingen vindt adequate anesthesie en pijnbestrijding plaats. De DEC is ervan overtuigd dat de dierproeven zo humaan mogelijk worden uitgevoerd. Er is geen sprake van belangwekkende milieueffecten.

10. De niet-technische samenvatting is een evenwichtige weergave van het project, zelfstandig leesbaar, beknopt en begrijpelijk geformuleerd.

D. Ethische afweging

Op basis van de onder C genoemde overwegingen komt de DEC tot de volgende ethische afweging.

Bij de dierproeven wordt op adequate wijze invulling gegeven aan de vereisten op het gebied van de vervanging, vermindering en/of verfijning van dierproeven (punt 7, 8 en 9). Het ongerief is voor 47% van de dieren licht, voor 38% van de dieren matig en voor 15% van de dieren gedurende korte tijd ernstig (punt 6).

Tegenover de nadelige gevolgen voor de dieren staat dat dit preklinisch onderzoek in muizen onmisbaar is bij het vertalen van de resultaten van fundamenteel kankeronderzoek en van observaties bij patiënten, naar nieuwe en verbeterde (combinatie)therapieën in de kliniek. De commissie classificeert het belang van de doelstelling van het project daarom als essentieel (punt 3). De doelstellingen acht de commissie zonder meer haalbaar (punt 4). De commissie is daarom van mening dat, in het licht van het essentiële belang van de beoogde resultaten van dit onderzoek, de onvermijdelijke nadelige gevolgen voor het welzijn van de dieren ethisch aanvaardbaar zijn. Aan de eis dat het belang van de experimenten op dient te wegen tegen het ongerief dat de dieren wordt berokkend, is voldaan.

E. Advies

1. Advies aan de CCD

- ☐ De DEC adviseert de vergunning te verlenen

2. Het uitgebrachte advies is gebaseerd op consensus.

Met vriendelijke groet,

[redacted]

Centrale Commissie Dierproeven

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Onze referentie
Aanvraagnummer
AVD301002016407
Bijlagen
2

Datum 28 januari 2016
Betreft Ontvangstbevestiging Aanvraag projectvergunning Dierproeven

Geachte [redacted]
Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 27 januari 2016.
Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD301002016407. 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).

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

Gegevens aanvrager

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

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

Gegevens verantwoordelijke uitvoering proces

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

Over uw aanvraag

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

Over uw project

Geplande startdatum:	15 februari 2016
Geplande einddatum:	15 februari 2021
Titel project:	Pre-clinical intervention studies in mice for prevention and treatment of cancer
Titel niet-technische samenvatting:	Preklinische interventiestudies in muizen voor kankerpreventie of behandeling
Naam DEC:	NKI
Postadres DEC:	t.a.v. [redacted]; Postbus 90203; 1006 BE; Amsterdam
E-mailadres DEC:	[redacted]

Betaalgegevens

De leges bedragen:	€ 1727,-
De leges voldoet u:	na ontvangst van de factuur

Checklist bijlagen

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

Ondertekening

Naam:	[redacted]
Functie:	[redacted]
Plaats:	I Amsterdam
Datum:	27 januari 2016

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

[redacted]

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

Onze referentie
Aanvraagnummer
AVD301002016407

Bijlagen
2

Datum 28 januari 2016
Betreft Factuur aanvraag projectvergunning Dierproeven

Factuur
Factuurdatum: 28 januari 2016
Vervaldatum: 27 februari 2016
Factuurnummer: 16700407
Ordernummer: Cost center 4050 / Project 002

Omschrijving	**Bedrag**
Betaling leges projectvergunning dierproeven Betreft aanvraag AVD301002016407	€

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

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Stichting Het Nederlands Kanker Instituut - Antoni van Leeuwenhoek ziekenhuis
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T 0900-28 000 28 (10 ct /min)

info@zbo-ccd.nl

Onze referentie
AVD301002015407

Uw referentie

Bijlagen
1

Datum 17 maart 2016
Betreft Beslissing Aanvraag projectvergunning dierproeven

Geachte [redacted]

Op 27 januari 2016 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project 'Pre-clinical intervention studies in mice for prevention and treatment of cancer' met aanvraagnummer AVD301002016407. Wij hebben uw aanvraag beoordeeld.

Beslissing
Wij keuren uw aanvraag goed op grond van artikel 10a van de Wet op de dierproeven (hierna: de wet). U kunt met uw project 'Pre-clinical intervention studies in mice for prevention and treatment of cancer' starten. De vergunning wordt afgegeven van 17 maart 2016 tot en met 15 februari 2021.

Voorwaarde
Aan deze vergunning zijn de voorwaarden verbonden zoals genoemd in de vergunning en hieronder toegelicht.

1) In artikel 4 lid 2 Richtlijn 2010/63/EU als in artikel 1d juncto artikel 10 lid 2 aanhef en onder sub a van de Wod is opgenomen dat, indien er verschillende methoden bestaan om een dierproef te verrichten, wordt gekozen voor de dierproef waarbij een zo gering mogelijk aantal dieren wordt gebruikt. Wij zijn van mening dat hieronder ook het aantal in voorraad gedode proefdieren valt. Wij hechten er daarom aan het aantal in voorraad gedode dieren te beperken.

U heeft in uw aanvraag aangegeven dat in principe zowel mannelijke als vrouwelijke dieren gebruikt worden. Echter per proef zal één geslacht geselecteerd worden. Daarnaast geeft u aan dat voor specifieke tumoren alleen mannelijke of vrouwelijke dieren gebruikt worden. Wij zijn van mening, dat indien tumoren enkel in mannen of vrouwen voorkomen, het acceptabel is dat onderzoek naar deze tumoren in het desbetreffende geslacht wordt uitgevoerd. U heeft echter niet onderbouwd voor welke andere tumoren het noodzakelijk zou zijn proeven in een enkel geslacht uit te voeren. Mannelijke en vrouwelijke dieren dienen daarom voor het overige in evenredige aantallen gebruikt worden. Indien gedurende het project blijkt dat er overige geslachts-specifieke effecten zijn, kunt u deze informatie als wijziging rapporteren aan de CCD. Deze rapportage kan voor de CCD aanleiding zijn om de voorwaarde van gelijk gebruik van beide geslachten te wijzigen of in te trekken. Deze voorwaarde is toegevoegd om het aantal in voorraad gedode dieren te beperken.

2) In uw aanvraag geeft u aan dat al lopende studies (waarvoor voor 18 december 2014 nog een positief DEC advies is afgegeven) niet zullen worden overgezet naar een eventuele CCD-vergunning. Nieuwe studies zullen wel worden uitgevoerd op de CCD-vergunning en niet op de

nog lopende DEC protocollen. Wij stellen vast dat er overlap is tussen oude nog lopende DEC protocollen en deze aanvraag. De dierproeven die onder de aanvraag vallen, maken integraal onderdeel uit van deze vergunning.

3) Uw aanvraag is een koepelaanvraag die gericht is op het onderzoeken van de effectiviteit van nieuwe therapieën tegen kanker. Alle onder deze koepelaanvraag uit te voeren interventiestudies vallen onder dezelfde doelstelling. Wij zijn van mening dat voor elk van de bijlagen dierproeven duidelijk beschreven is welke handelingen de dieren zullen ondergaan. Bovendien zijn de go/no go momenten tussen de verschillende dierproeven helder beschreven, is voor elk van de te gebruiken tumormodellen inzichtelijk gemaakt welk ongerief de dieren maximaal zullen ondergaan indien dieren een humaan eindpunt bereiken, wat de haalbaarheid van elk model is en wat de bijzonderheden voor elk model zijn.

Wij zijn daarnaast van mening dat het gecentraliseerd uitvoeren van de in deze aanvraag beschreven interventiestudies de haalbaarheid van dergelijke studies zal vergroten en uiteindelijk zal leiden tot vermindering en verfijning.

U verstrekt echter geen inzicht in de specifieke studies die zullen worden uitgevoerd en de specifieke onderzoeksvragen die zullen worden beantwoord. Wij begrijpen dat, gezien de aard van de aanvraag, het op dit moment nog niet mogelijk is inzichtelijk te maken welke tumoren en behandelingsvormen gedurende de looptijd van het project getest zullen worden. Dit betekent echter dat er op uitwerkingsniveau nog onduidelijkheden in het project zijn waarop niet beoordeeld kan worden.

Gezien de heldere uitwerking van de experimentele opzet, de te maken keuzes, het ongerief en de humane eindpunten zijn wij van mening dat er in dit geval ondanks het ontbreken van deze informatie wel een schade-baten analyse kan worden uitgevoerd, zoals vereist in artikel 10a2, lid 2d, van de Wod. Wij zijn tot de conclusie gekomen dat de schade in de vorm van pijn, lijden, angst of blijvende schade bij de dieren wordt gerechtvaardigd door het te verwachte resultaat met inachtneming van de ethische overwegingen, en op termijn voordelen kan opleveren voor de mens. Op basis hiervan hebben wij besloten deze aanvraag te vergunnen.

Wij hechten er wel aan inzicht te krijgen in hetgeen gedurende de studie onderzocht zal worden. U dient ons daarom jaarlijks schriftelijk te rapporteren welke interventiestudies er zijn uitgevoerd. Deze rapportage kan aanleiding zijn om de vergunning tussentijds te wijzigen zodanig dat toekomstige studies vooraf als wijzigingsaanvraag moeten worden ingediend. Voor de details van de voorwaarde, verwijzen wij u naar de vergunning.

Beoordeling achteraf
Na afloop van het project zal er een beoordeling plaatsvinden, zoals bedoeld in artikel 10a1 lid 1d en lid 3 van de wet. Meer informatie over de eisen bij een beoordeling achteraf vindt u in de bijlage.

Procedure
Bij uw aanvraag heeft u een advies van de Dierexperimentencommissie DEC-NKI gevoegd zoals ontvangen op 27 januari 2016. Bij de beoordeling van uw aanvraag is het advies van de DEC betrokken overeenkomstig artikel 10a lid 3 van de wet. Dit advies en de in de bijlage opgenomen beschrijving van de artikelen van de wet- en regelgeving liggen ten grondslag aan dit besluit. Wij nemen het advies van de Dierexperimentencommissie grotendeels over met uitzondering van bovenstaande afwijkingen. In aanvulling op het DEC advies hebben wij ook een algemene voorwaarde opgenomen in de vergunning.

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 gegevens in het colofon.

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).

De Centrale Commissie Dierproeven
namens deze

[redacted]

ir. G. de Peuter
Algemeen Secretaris

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

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

Projectvergunning

gelet op artikel 10a van de Wet op de dierproeven
Verleent de Centrale Commissie Dierproeven aan

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

deze projectvergunning voor het tijdvak 17 maart 2016 tot en met 15 februari 2021, voor het project 'Pre-clinical intervention studies in mice for prevention and treatment of cancer' met aanvraagnummer AVD301002016407, volgens advies van Dierexperimentencommissie DEC-NKI. Hierbij is afgeweken van het DEC-advies om te borgen dat mannelijke en vrouwelijke dieren in evenredige aantallen gebruikt worden, er geen overlap is met lopende DEC protocollen en om inzicht te krijgen in de interventiestudies die gedurende het project zullen worden uitgevoerd.

De functie van de verantwoordelijk onderzoeker is onderzoeker.
De aanvraag omvat de volgende bescheiden:

1. een aanvraagformulier projectvergunning dierproeven, ontvangen op 27 januari 2016
2. de bij het aanvraagformulier behorende bijlagen:
    a. Projectvoorstel, zoals ontvangen per digitale indiening op 27 januari 2016;
    b. Niet-technische Samenvatting van het project, zoals ontvangen per digitale indiening op 27 januari 2016;
    c. Advies van Dierexperimentencommissie, zoals ontvangen per digitale indiening op 27 januari 2016;

Naam dierproef	Diersoort	Aantal dieren	Ernst
Intervention protocol for recipient mice with transplantable tumors	Muizen	26.375	Licht 35% Matig 55% Ernstig 10%
Intervention protocol for genetically engineered mice with spontaneous or somatically induced tumors	Muizen	8.750	Licht 50% Matig 25% Ernstig 25%
Intervention protocol for recipient mice with metastatic tumors	Muizen	5.150	Licht 25% Matig 25% Ernstig 50%
Tolerability studies	Muizen	5.000	Ernstig (20%)
Pharmacological studies	Muizen	10.000	Licht (100%)

Na afloop van dit project wordt een beoordeling achteraf uitgevoerd. Deze beoordeling zal uiterlijk 15 mei 2021 plaatsvinden.

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

1) Mannelijke als vrouwelijke dieren moeten in evenredige aantallen gebruikt worden, met uitzondering van onderzoeksvragen waarbij tumoren onderzocht worden die enkel in mannen of vrouwen voorkomen. Indien gedurende het project blijkt dat er overige geslachts-specifieke effecten zijn, kunt u deze informatie als wijziging rapporteren aan de CCD. Deze informatie kan voor de CCD aanleiding zijn om bovenstaande voorwaarde van gelijk gebruik van beide geslachten te wijzigen of in te trekken. Indien voorafgaand aan de proeven al informatie in de literatuur beschikbaar is waaruit blijkt dat een model of proces geslachtsafhankelijk zou zijn, is het ook mogelijk om deze informatie te gebruiken om

wetenschappelijk te onderbouwen dat het gebruik van zowel mannelijke als vrouwelijke dieren zou leiden tot een grote toename van het benodigd aantal dieren en dit aan ons te rapporteren.

2) Daar waar er sprake is van overlap tussen de in deze vergunning vergunde dierproeven en eerder goedgekeurde DEC protocollen zullen de dieren en studies na het verlenen van de vergunning formeel onder deze vergunning gaan vallen. Hierdoor is er geen sprake meer van overlap.

3) De uitgevoerde interventiestudies dienen jaarlijks aan de CCD gerapporteerd te worden. In deze rapportage dient aandacht te worden besteed aan de voor elke behandelingsvorm specifieke onderzoeksvragen, de geteste behandelingsvormen, het type tumoren waarvoor behandelingsvormen getest zijn, de gebruikte modellen en het aantal dieren dat voor de specifieke onderzoeksvragen benodigd was. Deze informatie kan voor de CCD aanleiding zijn om de vergunning tussentijds te wijzigen zodanig dat nieuwe interventiestudies vooraf via een wijzigingsaanvraag ter toetsing aan de CCD voorgelegd dienen te worden. Het niet aanleveren van deze rapportage kan aanleiding zijn voor het intrekken van de vergunning. De eerste rapportage dient uiterlijk 17 maart 2017 te worden ingediend.

Algemene voorwaarde

4) In artikel 10, lid 1a 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 overleg met de IvD te melden bij de CCD. De CCD kan in een dergelijke situatie aan de vergunning nieuwe voorwaarden verbinden en gestelde voorwaarden wijzigen of intrekken.

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 van Economische Zaken 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 13c volgt dat het doden van dieren door een deskundig persoon moet worden gedaan, wat zo min mogelijk pijn, lijden en angst met zich meebrengt. De methode om te doden is vastgesteld in de Europese richtlijn artikel 6.
In artikel 13d 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.