	Inventaris Wob-verzoek W16-07S								
		wordt verstrekt				**weigeringsgronden**			
nr.	**document**	**reeds openbaar**	**niet**	**geheel**	**deels**	**10.1.c**	**10.2.e**	**10.2.g**	**11.1**
	NTS2015338								
1	Aanvraagformulier				x		x	x	
2	Projectvoorstel oud				x		x	x	
3	Niet-technische samenvatting oud			x					
4	Bijlage beschrijving dierproeven 1 oud				x		x	x	
5	Bijlage beschrijving dierproeven 2 oud				x		x	x	
6	DEC-advies				x		x	x	
7	Ontvangstbevestiging				x		x	x	
8	Mail vragen DEC 21-12-2015				x		x	x	
9	Reactie vragen DEC			x					
10	Mail reactie onderzoeker 22-12-2015				x		x	x	
11	Projectvoorstel herzien				x		x	x	
12	Bijlage beschrijving dierproeven 1 herzien				x		x	x	
13	Bijlage beschrijving dierproeven 2 herzien				x		x	x	
14	Niet-technische samenvatting herzien	x							
15	Advies CCD		x						x
16	Beschikking en vergunning				x		x	x	
17	Mail terugkoppeling DEC 28-1-2016				x		x	x	

AVD 105002015338

27 NOV 2015

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.zbo-ccd.nl of in de toelichting op de website.
- Of bel met 0900-2800028 (10 ct/min).

1 Gegevens aanvrager

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

☒ Ja > *Vul uw deelnemernummer in*	10500
☐ Nee > *U kunt geen aanvraag doen*	

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

Naam instelling of organisatie	Rijksuniversiteit Groningen
Naam van de portefeuillehouder of diens gemachtigde	[redacted]
KvK-nummer	1179037
Straat en huisnummer	A. Deusinglaan 1, [redacted]

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

Postbus	
Postcode en plaats	9713AV Groningen
IBAN	NL80ABNA0446049352
Tenaamstelling van het rekeningnummer	Rijksuniversiteit Groningen

1.4 Vul de gegevens in van de verantwoordelijke onderzoeker.

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

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

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

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

2 Over uw aanvraag

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

3 Over uw project

3.1	Wat is de geplande start- en einddatum van het project?	Startdatum	1 - 12 - 2015
		Einddatum	1 - 12 - 2020
3.2	Wat is de titel van het project?	To change the course of chemotherapy-induced mucositis	
3.3	Wat is de titel van de niet-technische samenvatting?	Het voorkomen of verminderen van ontsteking aan de darmwand veroorzaakt door chemotherapie.	
3.4	Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?	Naam DEC	DEC-RUG
		Postadres	A. Deusinglaan 1 [redacted]
		E-mailadres	secrdec.umcg@umcg.nl

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

☒ Nieuwe aanvraag Projectvergunning € 741,00 Lege

☐ Wijziging € Lege

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

☒ 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	Groningen
Datum	25 - 11 - 2015
Handtekening	[redacted]

Form
Project proposal

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

1 General information

1.1	Provide the approval number of the ‘Netherlands Food and Consumer Product Safety Authority’.	10500
1.2	Provide the name of the licenced establishment.	Rijksuniversiteit Groningen
1.3	Provide the title of the project.	To change the course of chemotherapy-induced mucositis

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.

Survival of children with cancer has increased significantly over the last decades, due to more intensive treatment protocols. The disadvantage of these more intensive chemotherapy treatment protocols is a higher frequency of side effects. One of these side effects is gastrointestinal mucositis (hereafter mucositis); a complex inflammatory reaction of the mucous membranes of the alimentary tract. Mucositis develops via different biological stages resulting in villus atrophy, ulceration and loss of barrier function, ending with spontaneous healing. The patients suffer from vomiting, abdominal pain, diarrhea, weight loss and are at increased risk of developing a bacteremia or sepsis. Mucositis leads to a lower quality of life and an increased morbidity and mortality. So far no interventions are found which prevents or to treat GI mucositis.

In this project we aim to change to course of chemotherapy-induced mucositis. To be able to achieve the aim, both fundamental and translational research is required.

Mucositis is a complex multifactorial process with many different established contributors (intestinal epithelium, immune system and nutrition). Based on these contributors a 5-phase model was developed for the pathobiology of oral mucositis.[1] This model is also the basis for the pathobiology of gastrointestinal mucositis. However, as one can imagine the environment in the intestine is markedly different from the oral environment, and thus the pathophysiology of gastrointestinal mucositis is probably (slightly) different. In short, the 5-phase model describes an initiation phase during which nuclear factor kappa B (NFκB) is activated by the formation of reactive oxygen species (ROS) and the occurrence of damage to the DNA. This is followed by the primary damage response phase during which messenger molecules / cytokines (among others: tumour necrosis factor α (TNFα)) are induced which lead to tissue inflammation and apoptosis. In the amplification phase the messenger molecules / cytokines are amplified due to positive feedback loops leading to increased inflammation and apoptosis. During the ulcerative phase the epithelial barrier integrity is lost due to the apoptosis and intestinal damage, which is spontaneously healed during the healing phase. However, in the gut there are other possible contributors which need to be explored for example the gut microbiota.[2,3] These microbiota have been reported to play a role in gastrointestinal mucositis in several studies so far.

In this project we will do fundamental research into a possible contributor which is not included in the 5-phase model, the gut microbiota.[2] The gut is in a continuous state of low grade infection due to the interactions between the gut microbiota and the immune system. Previous research has shown there is a change in microbial composition of the microbiota after chemotherapy, coinciding with gastrointestinal mucositis.[3] These changes in the microbiota potentially influence the inflammatory reaction seen during mucositis, since a change in composition of the gut microbiota would also alter the balance in the continuous state of low grade infection in the intestine. Interventions aimed to influence the composition of the gut microbiota could positively influence the course of mucositis by promoting a healthy composition of the microbiota, which could restore the normal state of low-grade inflammation in the intestine.

The second component we want to investigate in this project are interventions aimed to influence the inflammatory response and/or improve the intestinal barrier. The 5-phase model is mainly revolved around the inflammatory reaction present during mucositis. Drugs which influence this inflammatory reaction can potentially influence the course of gastrointestinal mucositis by reducing the damage done by the inflammation. An integral part of the damage done during mucositis is the ulceration phase, during which the patient suffers from the loss of the barrier function. Therapies which improve the barrier function by for example promoting proliferation, reducing apoptosis, improving cell junctions and/or preventing cellular changes, could reduce or prevent the damage to the intestinal barrier which is the main problem for the patient.
The last component we will investigate are nutritional based interventions. In previous research performed by our group the absorption of different components of the nutrition during mucositis were investigated.[4] Nutrition is known to be able to influence the barrier function of the intestine during mucositis and other intestinal complications. In this study we will continue investigations into potential nutritional interventions which improve the barrier function and could help speed up the recovery after the ulceration phase.
A strength of this study is the potential of the different components of the study to influence each other. The nutrition is for example known to influence the composition of the microbiota, which could lead to double beneficial effects.

The model we developed and will use in this project is well validated and suitable to test interventions based on the results of our basic research. Therefore, during this project translational research will be performed to study whether we can change the course of chemotherapy-induced mucositis.

(1) [redacted]
(2) [redacted]
(3) [redacted]
[redacted]
(4) [redacted]
[redacted]

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 find prevention or treatment strategies which change the course of severe mucositis. To achieve this objective several potential strategies will be investigated namely, the gut microbiota, therapies/prophylaxis and nutrition. Also the mechanism behind the interventions will be investigated.

We deem the aim of this project feasible within the duration of this project. In previous projects the chemotherapy-induced mucositis rat model which we will use in this project was developed. The model is now well validated and there is a tremendous amount of experience with the model.[1-4] The project consists of different research avenues which will be investigated simultaneously with good communication between the different contributors. The combination of the different areas of research and combination of knowledge gained within each area will help us achieve the aim of the project.

3.3 Relevance

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

Previous research has shown that 55% of the children receiving chemotherapy develop intestinal mucositis. On top of that when patients were asked the most debilitating side-effect of chemotherapy, symptoms of mucositis were mentioned the most. The overall consequences of the mucositis not only involves quality of life after chemotherapy courses, but probably also decreases survival due to treatment reduction, treatment delay, decreased nutritional state, which on its own is associated with treatment complications and increased mortality. Up until now there are no interventions in place which prevent or improve the course of mucositis, which we want to change with this project.

(1) [redacted]
(2) [redacted]
(3) [redacted]
(4) [redacted]

3.4 Research strategy

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

This projects aims to change the course of chemotherapy-induced mucositis and their mechanism of action. Different potential prevention or treatment strategies will be assessed in a chemotherapy-induced mucositis rat model.

The first strategy is to investigate the relevance of intervening at the level of the gut microbiota. The pathophysiology of the possible relationship between the GI microbiota and mucositis has been extensively reviewed in a paper of our research group. However, little research to investigate the relationship has been done until this point. We investigated the microbiota of pediatric acute myeloid leukemia (AML) patients, which demonstrated a significant decrease in the total number and a change in composition of the fecal bacteria. This result was replicated in a rat model of chemotherapy-induced mucositis by our group and by others. The relationship between the microbiota and the development and recovery of mucositis and possible interventions is the first possible strategy investigated here. Possible interventions for the microbiota are among others antibiotics to limited potential pathogenic bacteria, pro- and prebiotics to promote a ‘healthy’ population of the microbiota or a combination of the different interventions.

Secondly, interventions aimed to influence the inflammatory response and/or improve the intestinal barrier will be investigated. Previous research has shown the beneficial effects of inhibition of several cytokines (among others TNFα and IL-1β).[1] Here, we will continue to investigate drugs which influence the inflammatory response during mucositis and further elucidate important components of the inflammatory response during mucositis. [redacted].[2] Another important feature in the previously mentioned 5-phase model is the disruption of the intestinal barrier during the ulceration phase. Interventions aimed to improve the intestinal barrier will be investigated in this study. One approach to improve the barrier function is to limit cell death, this can be accomplished to reduce apoptosis or via increased proliferation of the intestinal epithelial cells. Another approach is to improve the barrier function by interventions aimed at the cellular junctions. Increasing the amount or strength of the cellular junctions (for example tight junctions) between the epithelial cells, the disruption of the intestinal barrier might be prevented or limited. A possible mechanism to upregulate the tight junction proteins is stimulation of cannabinoid type 2 receptor which was investigated by Yang et al.[3]

A third possible strategy are interventions based on the nutrition. Previous research in our group has focused on feeding strategies based on parental nutrition or tube feeding strategies and the uptake of nutrients. In this study we will continue investigating optimal feeding strategies, on top of that we will also investigate nutritional interventions focused on supplementation of the intake. [redacted]

The three previously mentioned strategies are closely related, as the interventions investigated could also have an effect on other components. Nutrition is known to influence the microbiota composition in the gut and there is a link between the microbiota and the inflammatory response present, as the intestine is in a constant state of low grade inflammation due to interactions between microbiota and immune system. Therefore, combinations of effective interventions could be investigated to show whether the combination has an additive or synergistic effect on the course of severe mucositis.

To investigate the mechanism behind successful strategies we would like to have knock-outs available. The availability of knock-out rats is far from complete compared to mice. Therefore, we would like to transpose our chemotherapy-induced mucositis rat model into a mouse model to be able to investigate proposed mechanisms for the interventions. As described above, the interaction between the gut microbiota and the immune system is important for the development of mucositis. Therefore, we will study knock out models of genes encoding for receptors which are important for the interaction of this interaction (for example [redacted]). On the other hand the inflammatory response could be hypothesized to be the major contributor to the development of mucositis and a knock-out of a central gene (for example [redacted]) could reduce the severity of the mucositis. More genes of interest will be decided during the course of the study, depending on new literature and results of above mentioned studies.

The three different research strategies will all be started simultaneously and continue over the course of the entire project. The exact planning of these experiments is hard to predict since the outcomes of earlier experiments taken into account in the development and planning of new experiments. The development of the mouse model will most likely start in year two.

(1) [redacted]
(2) [redacted]
(3) [redacted]

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

The different components of the project will all employ the rat model of chemotherapy-induced mucositis which is a well validated model that has been developed and further employed in previous projects.[1-4] The last part of the project which tries to elucidate the mechanisms of different interventions will be performed in both our established rat model and a mouse model which is to be developed. The mouse model will be developed to have knock-outs available since in rats the knock-outs are limited.

For the first component of the study the chemotherapy-induced rat model of mucositis will be employed during which the relationship between the mucositis and the gut microbiota will be elucidated. To investigate this relationship different experiments will be performed during which (part of) the microbiota is depleted using antibiotics, bacterial strains will be added using probiotics, prebiotics will be given to modulated the composition of the gut microbiota or the effect of the gut microbiota will be mimicked.

The second component aims to prevent or elevate the mucositis by improving of cell survival, limiting inflammation or via other mechanisms. During these experiments the animals will be (prophylactically) treated with drugs which for example could improve cell survival, promote cell proliferations, limit apoptosis or limit inflammation.

The third component aims to intervene in the nutrition. [redacted]

The chemotherapy-induced mucositis mouse model will assess mechanisms of successful strategies which require knock-outs or other tests currently unavailable in rats. The development of the mouse model will most likely start in year two.

(1) [redacted]

(2) [redacted]

(3) [redacted]

(4) [redacted] WJ, Stellaard F, Verkade HJ, Rings EH. Reduced absorption of long-chain fatty acids during methotrexate-induced gastrointestinal mucositis in the rat. Clin Nutr 2013 Jun;32(3):452-459.

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

The components all have the goal to change the course of severe mucositis and the interventions are designed to target different factors which could influence the course of mucositis. However, the influence of each of the component is not limited to the component itself. Changes in nutrition also have an influence on the composition of the microbiota and could possibly also improve cell survival. The composition of the microbiota is also known to influence the inflammation in the intestine. Therefore, the different components of the project are closely linked together.

Interventions which improve the course of chemotherapy-induced mucositis will be selected and interventions combined if possible to investigate possible synergistic effects.

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	Chemotherapy-induced mucositis rat model
2	Chemotherapy-induced mucositis mouse model
3	
4	
5	
6	
7	

8	
9	
10	

Format
Niet-technische samenvatting

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

1 Algemene gegevens

1.1	Titel van het project	Het voorkomen of verminderen van ontsteking van de darmwand veroorzaakt door chemotherapie.
1.2	Looptijd van het project	01-12-2015 tot 01-12-2020
1.3	Trefwoorden (maximaal 5)	Chemotherapie, darmwandontsteking, kanker, mucositis

2 Categorie van het project

2.1 In welke categorie valt het project.

U kunt meerdere mogelijkheden kiezen.

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

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3 Projectbeschrijving

3.1	Beschrijf de doelstellingen van het project (bv de wetenschappelijke vraagstelling of het wetenschappelijk en/of maatschappelijke belang)	De overleving van kinderen met kanker is de laatste decennia enorm verbeterd, dit door zwaardere behandelmethodes. Het nadeel van deze zwaardere chemotherapie is dat er vaker bijwerkingen zijn. Een van de belangrijkste bijwerkingen van chemotherapie is een ontsteking van de darmwand, genaamd mucositis. Mucositis zorgt voor diarree en buikpijn. Als de diarree te ernstig wordt, kan de geplande behandeling met chemotherapie niet meer uitgevoerd worden. Dit beïnvloedt de kans op overleving. Bovendien verlaagt mucositis de kwaliteit van leven van het kind met kanker dramatisch. In dit project willen we proberen mucositis te voorkomen of sneller te genezen. Dit doen we door ons te richten op verschillende mogelijke therapieën. We willen de invloed van de darmbacteriën op de mucositis onderzoeken en kijken of deze gebruikt kunnen worden voor een therapie. Tevens gaan we onderzoek doen naar farmaca die ervoor kunnen zorgen dat het darmepitheel kan overleven en/of de ontsteking in de darm minder wordt. Als laatste wordt onderzocht of met toevoegingen aan de voeding de ernst van mucositis kan worden verminderd en het herstel verbeterd.
3.2	Welke opbrengsten worden van dit project verwacht en hoe dragen deze bij aan het wetenschappelijke en/of maatschappelijke belang?	In dit project willen we nieuwe therapieën vinden die het ziekteproces kunnen verminderen van mucositis die ontstaat door chemotherapie. Uit eerder onderzoek bleek dat meer dan de helft van de kinderen die chemotherapie kregen mucositis ontwikkelde. Daarbij gaven patiënten aan dat ze de symptomen die ontstaan door mucositis ervaren als de ergste bijwerking van chemotherapie. Doordat mucositis ook de overleving kan beïnvloeden door noodzakelijke veranderingen in het behandelschema, zou een therapie die mucositis kan voorkomen of het herstel kan versnellen een enorme verlichting zijn voor de patiënten. De uitkomsten uit dit project kunnen gebruikt worden om nieuwe therapieën bij mensen te ontwikkelen.
3.3	Welke diersoorten en geschatte aantallen zullen worden gebruikt?	Tijdens de vier jaar van dit project zijn naar schatting 720 ratten nodig (drie experimenten per jaar met elk 48 ratten). Ook willen we het model opzetten in muizen. De belangrijkste reden hiervoor is dat er in muizen stammen beschikbaar zijn die één gen missen (knock-outs). Die kunnen gebruikt worden om te bewijzen hoe de nieuwe therapie werkt. Omdat het muismodel nog ontwikkeld moet worden is het lastig om een schatting te geven van het aantal. De experimenten met muizen zullen echter vooral uitgevoerd worden als succesvolle therapieën zijn ontwikkeld in het ratmodel. We schatten dat er 360 muizen nodig zijn voor het opzetten en uitvoeren van de experimenten.
3.4	Wat zijn bij dit project de verwachte negatieve gevolgen voor het welzijn van de proefdieren?	Het grootste negatieve effect voor de proefdieren is het onder narcose toegediend krijgen van een chemotherapeuticum (bijv. methotrexaat) en de daaruit volgende mucositis. Hierdoor zijn de dieren vier dagen ziek (minder eten en diarree). Daarna herstellen de dieren van de mucositis. Andere negatieve gevolgen zijn het onder narcose afnemen van bloed, vasten voor een test om de opname uit de darm te testen en een druppetje bloed afnemen als onderdeel darmopname test.
3.5	Hoe worden de dierproeven in het project ingedeeld naar de verwachte ernst?	Matig
3.6	Wat is de bestemming van de dieren na afloop?	De dieren zullen worden geofferd aan het einde van de proef. Dit is nodig om de ernst van de mucositis te beoordelen en om darminhoud te verzamelen zodat we de bacteriën in de darm kunnen onderzoeken.

[

4 Drie V's

4.1	**Vervanging** Geef aan waarom het gebruik van dieren nodig is voor de beschreven doelstelling en waarom proefdiervrije alternatieven niet gebruikt kunnen worden.	Mucositis is een multifactorieel proces. Er zijn zoveel verschillende onderdelen in het lichaam die er invloed op hebben (darmwand, immuunsysteem, voeding, darmflora) dat dit niet in vitro (d.w.z. in weefsel buiten het lichaam) nagebootst kan worden. Bovendien is het onderzoek te invasief om het in de humane situatie uit te voeren.
4.2	**Vermindering** Leg uit hoe kan worden verzekerd dat een zo gering mogelijk aantal dieren wordt gebruikt.	Voor elk experiment zal het aantal benodigde dieren met statistische analyses beperkt worden. Waar mogelijk worden methoden gebruikt waarbij het offeren van de dieren niet nodig is. Hetzelfde dier kan dan op meerdere momenten gemeten worden.
4.3	**Verfijning** Verklaar de keuze voor de diersoort(en). Verklaar waarom de gekozen diermodel(len) de meest verfijnde zijn, gelet op de doelstellingen van het project.	Het ratmodel dat we in deze studie zullen gebruiken, is goed gevalideerd. Het is een model dat vaak gebruikt wordt bij onderzoek naar mucositis door chemotherapie. Ook is bekend dat de ratten in dit model herstellen van de chemotherapie. Dit is in andere modellen die gebruikt worden niet het geval.
	Vermeld welke algemene maatregelen genomen worden om de negatieve (schadelijke) gevolgen voor het welzijn van de	Het model is zo ingericht dat de ratten herstellen van de chemotherapie die gegeven wordt. Tijdens het toedienen van de chemotherapie zullen de dieren onder narcose gebracht worden om zo het ongerief voor de dieren te verminderen. De experimenten worden uitgevoerd door bevoegd en competent personeel.

[

proefdieren zo beperkt mogelijk te houden.

5 In te vullen door de CCD

Publicatie datum

Beoordeling achteraf

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'.	10500
1.2	Provide the name of the licenced establishment.	Rijksuniversiteit Groningen
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 1 Type of animal procedure: Chemotherapy-induced mucositis rat model

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

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

To assess the course of chemotherapy-induced mucositis a rat model was developed previously in our department.[1] To be able to investigate the chemotherapy-induced gastrointestinal mucositis (hereafter mucositis) the rats in our model receive an injection of a chemotherapeutic agent (for example methotrexate (MTX), irinotecan or other chemotherapeutics) after which the mucositis can be investigated. Initially the focus will be on MTX as the chemotherapeutic agent, later on other chemotherapeutics will be investigated as well since the effect of interventions might be different depending on the

mechanism of action of the chemotherapeutic agent. During a bout of mucositis the rats will develop diarrhoea, reduce their food intake and as such reduce in body weight. These parameters will be measured throughout the whole experiment.
To study different feeding strategies currently used in patients (parental feeding and tube feeding) a rat model with a catheter into the vena jugularis or the duodenum was developed. These rats will receive surgery during which they received a permanent catheter in the duodenum or vena jugularis which will be subcutaneously tunnelled to the head, where attachment to the swivel system is possible. The swivel system is used to continuously infuse parenteral or tube feeding. After the operation which places the catheter the rats receive analgesics for 24 hours and are enabled to recover for seven days. The dose of the chemotherapeutics in this model is lowered since the animals are under increased stress due to the catheter.

Primary outcome measures:

- Histology of the intestine: To determine the severity of mucositis, the histology of the intestine will be investigated. The histology will be measured on time points of interest when animals are sacrificed.
- Blood citrulline levels: To reduce the amount of animals needed the blood levels of citrulline and/or other (blood) markers will be measured during the experiment. Citrulline is an amino-acid almost exclusively produced by enterocytes, the blood levels of citrulline are a marker for the enterocyte mass in the intestine.
- Gut microbiota: To investigate the gut microbiota, intestinal content will be collected in which the microbiota will be measured. However, this is only possible after animals are sacrificed. Therefore, faeces will be collected to investigate the gut microbiota during the experiment.
- Glucose absorption: Before the rats will be sacrificed they will be fasted overnight and a glucose absorption test will be performed, this test is performed to test the intestinal function.

(1) [redacted]

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

In general the previously mentioned chemotherapy-induced rat model will be employed to investigate the effect of different interventions before and/or after administration of the chemotherapeutic agent. The mentioned model is optimized with the use of methotrexate as chemotherapeutic agent. When other chemotherapeutic agents will be used the dose is based on literature and optimized in pilots.

Intervention studies will be carried out with:

- Anti-, Pro-, pre- or synbiotics. These are interventions aimed to enlarge the bacterial population of the microbiota and/or to change its composition. Antibiotics will be aimed to reduce the populations of potentially pathogenic bacteria, these can be given in the food/drinking water or via oral gavage. Probiotics are one or more bacterial strains which are given via oral gavage, prebiotics are substances [redacted]) which can be given in the food/drinking water or via oral gavage and synbiotics are a combinations of the two. Depending on the aim of the experiment these will be given once or multiple times during the experiment.
- Drugs aimed to influence the inflammatory response. These drugs will most likely be given via an (intravenous, intraperitoneal or subcutaneous) injection, depending on the optimal route of the intervention, as a prophylaxis or therapy. With these studies we aim to influence the inflammatory response in a beneficial way to limit the damage done during mucositis or to fasten the recovery ([redacted]).
- Drugs targeting the intestinal barrier. Drugs improving the intestinal barrier will also most likely be given via an injection, although certain drugs could potentially be given via oral gavage or in the food/drinking water, given as prophylaxis or therapy. These drugs aim to prevent or limit the disruption of the intestinal barrier during the ulceration phase of mucositis ([redacted]

- Nutrition. Nutritional interventions include both feeding strategies and supplementation to food/water. [redacted]
- [redacted]

The chemotherapeutic agent will be administered under general anaesthesia and the rats are followed during the development and recovery of mucositis. Throughout the whole experiment body weight, intake and the presence of diarrhoea are measured. During the experiment blood samples will be taken daily or every other day to measure blood levels of citrulline and/or other biomarkers. Faecal samples will be collected to investigate the gut microbiota during the experiment. Animals will be sacrificed on points of interest which depend on the aim of the experiment.

During some experiments animals might receive two cycles of chemotherapy to investigate the effect of successful interventions during a second cycle of chemotherapy. The effect of successful intervention during two cycles will be investigated since patients also receive multiple during the treatment of their tumour.

The night before the animals are sacrificed the animals are fasted since this is needed to perform a glucose absorption test to investigate the function of the intestine. For this test the animals receive a bolus of labelled glucose via oral gavage and the blood glucose levels will monitored via repeated collection of blood via the tail vein.

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

Before each experiment a sample size calculation based on a power assessment with historical data from our laboratory (expected effect size and standard deviation of histology, blood citrulline levels, etc) will be performed to determine the group size of the animals. To reduce the amount of animals needed, the course of chemotherapy-induced mucositis will in general be monitored by blood citrulline levels, while histology is performed on a time point of interest.

B. The animals

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

We chose wild-type Wistar male rats as animal model in this project since in preceding projects a chemotherapy-induced rat model was developed. This is a validated model and the model is very suitable for the experiments planned in the present project. The model is optimized for male rats during the development of the model, which means the dose in this model is most likely not optimized for female rats. On top of that, the fact that mucositis is a multifactorial in their development adding another variable (male vs female) into the equation would increase the variation too much.

The rats in our experiments will be purchased from commercial sources both from inside and outside of the EU. The life stage of the animals is post-weaning and young adults depending on the aim of the experiment. We estimate that approximately 8 rats are required for each experimental group in which mucositis is induced. The exact number of rats needed for each experiment will be calculated with a sample size calculation based on historical data from our laboratory and a power assessment. With these group sizes, the estimation that each experiment has 3 groups with 2 time points and the estimation of 3 experiments each year of the five years of this project leads to an estimated number of 720 animals needed.

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.

Replacement: The goal of this project is to ameliorate the course of chemotherapy-induced mucositis. Mucositis is a multifactorial process which involves many different established contributors (intestinal epithelium, immune system and nutrition) which are hard to mimic in vitro. On top of that we will investigate the role of the intestinal microbiota. Therefore, an accurate in vitro model which combines all these factors is impossible to accomplish. The experiments are clearly too invasive to be able to investigate them in the human situation. Therefore, an animal model of chemotherapy -induced mucositis is needed.

Reduction: Experiments during which animals are sacrificed at two or more time points compared to the control group(s) will, when the experimental aim allows this, not be measured on each time point since the control animals do not change during the experiment. Also parameters which do not require the animals to be sacrificed are assessed whenever appropriate, for example citrulline in blood samples and faeces samples.

Refinement: The animal model employed in this project is a well validated model in which the development and recovery of mucositis can both be studied. Anaesthesia and analgesia will be used whenever appropriate to minimize stress and discomfort.

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

The animals are handled often to minimize stress and discomfort during weighing or while being transferred. On top of that, animals are monitored frequently to identify potential discomfort as soon as possible. Anaesthesia and analgesia are given whenever needed. Potential environmental-polluting waste will be collected appropriately.

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.

Several possible interventions are investigated which could potentially ameliorate the course of severe mucositis. The role of microbiota during the development and recovery of chemotherapy-induced mucositis has not yet been extensively investigated. The research that has been done so far showed potential but due to flaws in the experimental design results are not solid enough to allow direct translation to humans. Some studies report nutritional

interventions. However, these studies have so far focused on amino acid supplementation, other nutrients or combinations have hardly been studied.

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.

The animals will be housed individually. This is needed since group housing increases stress and in the model the increased stress leads to worsening of the symptoms. In turn this also leads to more variability between rats. Also the catheterized animals require individual housing due to the connection to a swivelsystem.

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.

The rats will be anesthetized with Isoflurane/oxygen by or under supervision of experienced employees. Analgesia will be used after the catheterization and whenever needed at other moments.

I. Other aspects compromising the welfare of the animals

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

After administration of the chemotherapeutic agent the rats will have 4 days of lowered food intake, weight loss (<10%), diarrhoea and general sickness.

Also in the catheter model there is increased stress on the animals, which potentiates effects of mucositis. Therefore, the dose of chemotherapeutics is lowered in this model.

Explain why these effects may emerge.

The goal of our project is to change to course of chemotherapy-induced mucositis, the symptoms of mucositis are an impaired uptake of nutrients and a general sickness. Due to an impaired uptake of nutrients more water will be attracted to the faeces which results in diarrhoea. Combined the general sickness and diarrhoea lead to weight loss.

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

Chemotherapy will be given at a dose which ensures the mucositis is self-limiting as it is in patients. This dose is optimized to minimize severity and suitable to investigate mucositis. The interventions which are investigated are aimed to prevent occurrence or minimise severity of the mucositis.

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.

When signs of severe complications, like severe weight loss (>15%) and general severe malaise (behavior, fur, hygiene) the rats will be sacrificed due to humane endpoints. After injection with the chemotherapeutic agent the rats will be investigated daily.

Indicate the likely incidence.

<5% of the animals receiving chemotherapy.

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’).

Non-recovery: collection of organs after being sacrificed
Mild: individual housing, transport during experiments, oral gavage, collection of blood from tail vein, injection of intervention (IV,IP,SC), dexa-scan or nuclear magnetic resonance spectroscopy, faecal transfer, repeated collection of blood during glucose absorption test, fasting and continuous infusion of parenteral or tube feeding.
Moderate: Administration of chemotherapy/NaCl via injection, experiencing mucositis and placement of permanent catheter into the vena jugularis or duodenum.
The overall expected level of discomfort is moderate. The animals will be closely monitored after administration of chemotherapy until the end of the experiment.

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.

To be able to investigate the histology of the intestine to evaluate the mucositis and its recovery and to collect the content of the gastrointestinal tract to

evaluate the gut microbiota. Other organs are also investigated and harvested depending on the research question.

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

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

☒ Yes

Appendix
Description animal procedures

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1 General information

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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: 2 Type of animal procedure: Chemotherapy-induced mucositis mouse model

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.

Since the aim with the mouse model is to replicate the results from the rat model to have knock-outs available, the mouse model will be designed to be as similar as possible to the rat model as possible given the differences between the two species. Therefore, there will be overlap between the outcome measures and interventions possibly performed in both models.

The main reason to develop a chemotherapy-induced mucositis mouse model is the availability of transgenic mouse models enabling mechanistic studies of causes of mucositis. The mouse model will be developed based on available literature combined with experience we have with the rat model. Based on literature a suitable mouse strain will be selected and an approximate chemotherapy dose will be determined to be given IV and in a single dose. The dose will be optimised in several experiments to ensure minimal severity for the mouse, combined with a suitable model to investigate mucositis.

To be able to investigate the chemotherapy-induced gastrointestinal mucositis (hereafter mucositis) the mice in our model will receive an injection of a chemotherapeutic agent (for example methotrexate, irinotecan or other chemotherapeutics) after which the mucositis can be investigated. During a bout of mucositis the mice will develop diarrhoea, reduce their food intake and as such reduce in body weight. These parameters will be measured throughout the experiments.

Primary outcome measures:

- Histology of the intestine: To determine the severity of mucositis, the histology of the intestine will be investigated. The histology will be measured on time points of interest when animals are sacrificed.
- Blood citrulline levels: To reduce the amount of animals needed the blood levels of citrulline and/or other (blood) markers will be measured during the experiment. Citrulline is an amino-acid almost exclusively produced by enterocytes, the blood levels of citrulline are a marker for the enterocyte mass in the intestine.
- Gut microbiota: To investigate the gut microbiota, intestinal content will be collected in which the microbiota will be measured. However, this is only possible after animals are sacrificed. Therefore, faeces will be collected to investigate the gut microbiota during the experiment.
- Glucose absorption: Before the rats will be sacrificed they will be fasted overnight and a glucose absorption test will be performed, this test is performed to test the intestinal function.

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

When the mice model is successfully developed and optimized, the model will be used to investigate the effect of different interventions before and/or after administration of the chemotherapeutic agent.

The main objective in the mouse model will be to confirm improved results found in the rat model and to investigate a genetic involvement in the mechanism of action. Intervention studies will be carried out with:

- Knock-out genes. To investigate the proposed mechanisms of successful interventions an important tool is the use of transgenic mice. The interaction between the gut microbiota and the immune system is important for the development of mucositis. Therefore, we will study knock out models of genes encoding for receptors which are important for the interaction of this interaction ([redacted]). On the other hand the inflammatory response could be hypothesized to be the major contributor to the development of mucositis and a knock-out of a central gene ([redacted] could reduce the severity of the mucositis. More genes of interest will be decided during the course of the study, depending on new literature and results of above mentioned studies.

Since the mouse model will be employed to investigate genetic involvement in the mechanism of action of the interventions which were successful, we will need to confirm whether these interventions are successful in mice as well.

- Pro-, pre or synbiotics. These are interventions aimed to enlarge the bacterial population of the microbiota and/or to change its composition. Probiotics are one or more bacterial strains which are given via oral gavage, prebiotics are substances ([redacted]) which can be given in the nutrition or via oral gavage and synbiotics are a combinations of the two. Depending on the aim of the experiment these will be given once or multiple times during the experiment.

- Drugs aimed to influence the inflammatory response. These drugs will most likely be given via an (intravenous, intraperitoneal or subcutaneous) injection, depending on the optimal route of the intervention, as a prophylaxis or therapy. With these studies we aim to influence the inflammatory response in a beneficial way to limit the damage done during mucositis or to fasten the recovery ([redacted]).
- Drugs targeting the intestinal barrier. Drugs improving the intestinal barrier will also most likely be given via an injection, although certain drugs could potentially be given via oral gavage or in the food/drinking water, given as prophylaxis or therapy. These drugs aim to prevent or limit the disruption of the intestinal barrier during the ulceration phase of mucositis ([redacted]).
- Nutrition. Nutritional interventions are focussed on supplementation of nutrients to food/water. [redacted]
- [redacted]

The chemotherapeutic agent will be administered under general anaesthesia and the mice are followed during the development and recovery of mucositis. Throughout the whole experiment body weight, intake and the presence of diarrhoea are measured. Blood will be drawn to assess the plasma citrulline levels and faecal samples will be collected to investigate the gut microbiota during the experiment. Animals will be sacrificed on points of interest which depend on the aim of the experiment.

During some experiments animals might receive two cycles of chemotherapy to investigate the effect of successful interventions during a second cycle of chemotherapy. The effect of successful intervention during two cycles will be investigated since patients also receive multiple during the treatment of their tumour.

The night before the animals are sacrificed the animals are fasted since this is needed to perform a glucose absorption test to investigate the function of the intestine. For this test the animals receive a bolus of labelled glucose via oral gavage and the blood glucose levels will monitored via repeated collection of blood via the tail vein.

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

Before each experiment a sample size calculation based on a power assessment with historical data from our laboratory (expected effect size and standard deviation of histology, blood citrulline levels, etc) will be performed to determine the group size of the animals. To reduce the amount of animals needed, the course of chemotherapy-induced mucositis will in general be monitored by blood citrulline levels, while histology is performed on a time point of interest.

B. The animals

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

Both WT and transgenic male mice will be used in this mouse model of chemotherapy-induced mucositis. The strain and life stage of the mice will be determined during the development of the mice model. Male mice will be used to keep rat and mouse model as similar as possible. Since mucositis is a multifactorial in their development adding another variable (male vs female) into the equation would increase the variation too much. The mice will be purchased from commercial sources both from inside and outside of the EU. The life stage of the animals is post-weaning and young adults depending on the aim of the experiment.

The exact number of mice needed for each experiment will be calculated with a sample size calculation based on historical data from our laboratory and a

power assessment. Since the model still needs to be developed an estimation of the amount of mice needed in total is hard and this depends on the number of experiments needed to optimize the model. We estimate that in total 360 mice are needed, 120 of which for the development of the mice model.

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.

Replacement: The goal of this project is to ameliorate the course of chemotherapy-induced mucositis. Mucositis is a multifactorial process which involves many different established contributors (intestinal epithelium, immune system and nutrition) which are hard to mimic in vitro. On top of that we will investigate the role of the intestinal microbiota. Therefore, an accurate in vitro model which combines all these factors is impossible to accomplish. The experiments are clearly too invasive to be able to investigate them in the human situation. Therefore, an animal model of chemotherapy-induced mucositis is needed. The availability of transgenic rats is very limited. To be able to investigate the involvement of specific genes in successful interventions a mice model would be necessary.

Reduction: Experiments during which animals are sacrificed at two or more time points compared to the control group(s) will, when the experimental aim allows this, not be measured on each time point since the control animals do not change during the experiment. Also parameters which do not require the animals to be sacrificed are assessed whenever appropriate, for example citrulline in blood samples and faeces samples.

Refinement: The model will be designed to minimize discomfort and stress for the animals. Anaesthesia and analgesia will be used whenever appropriate to minimize stress and discomfort.

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

The animals are handled often to minimize stress and discomfort during weighing or while being transferred. On top of that, animals are monitored frequently to identify potential discomfort as soon as possible. Anaesthesia and analgesia are given whenever needed. Potential environmental-polluting waste will be collected appropriately.

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.

Several possible interventions are investigated which could potentially ameliorate the course of severe mucositis. The role of microbiota during the development and recovery of chemotherapy-induced mucositis has not yet been extensively investigated. The research that has been done so far showed potential but due to flaws in the experimental design results are not solid enough to allow direct translation to humans. Some studies report nutritional interventions. However, these studies have so far focused on amino acid supplementation, other nutrients or combinations have hardly been studied.

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.

The animals will be housed individually. This is needed since group housing increases stress and in the model the increased stress leads to worsening of the symptoms. In turn this also leads to more variability between mice.

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.

The mice will be anesthetized with Isoflurane/oxygen by or under supervision of experienced employees. Analgesia will be used if needed.

I. Other aspects compromising the welfare of the animals

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

After administration of chemotherapeutic agent the mice will have several days of lowered intake, weight loss (<10%), diarrhoea and general sickness. We expect the adverse effects of WT mice welfare be similar to the adverse effects of the rat model. Genetic effects present in transgenic mice might possibly compromise welfare of the animals. However, these effects are impossible to describe now since missing gene will be determined based on results of experiments performed in this project. The mice used in this project will be already established transgenic mice strains. How these transgenic strains fare after the administration of chemotherapy could go both ways, depending on the missing gene and the mechanism we want to investigate. A gene maybe need to be inhibited/induced to improve the course of mucositis, a transgenic mice might genetically have an beneficial/worse outcome.

Explain why these effects may emerge.

The goal of our project is to change to course of chemotherapy-induced mucositis, the symptoms of mucositis are an impaired uptake of nutrients and a general sickness. Due to an impaired uptake of nutrients more water will be attracted to the faeces which results in diarrhoea. Combined the general sickness and diarrhoea lead to weight loss.

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

The interventions which are investigated are aimed to prevent occurrence or minimise severity of the mucositis. Also chemotherapy will be given at a dose which ensures the mucositis is self-limiting as it is in patients.

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.

When signs of severe complications, like severe weight loss (>15%) and general severe malaise (behavior, fur, hygiene) the mice will be sacrificed due to humane endpoints. After chemotherapy-injection the mice will be investigated daily.

Indicate the likely incidence.

<5% of the mice which receive chemotherapy

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').

Non-recovery: collection of organs after being sacrificed
Mild: individual housing, transport during experiments, oral gavage, collection of blood from tail vein, injection of intervention (IV,IP,SC), dexa-scan or nuclear magnetic resonance spectroscopy, faecal transfer, repeated collection of blood during glucose absorption test and fasting
Moderate: Administration of chemotherapy/NaCl via injection and experiencing mucositis.
The overall expected level of discomfort is moderate. The animals will be closely monitored after administration of chemotherapy until the end of the experiment.

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.

To be able to investigate the histology of the intestine to evaluate the mucositis and its recovery and to collect the content of the gastrointestinal tract to evaluate the gut microbiota. Other organs are also investigated and harvested depending on the research question

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

10 december 2014

Format DEC-advies

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

A. Algemene gegevens over de procedure

1. Aanvraagnummer: (Interne RuG code **8024**)
2. Titel van het project: **To change the course of chemotherapy-induced mucositis.**
3. Titel van de NTS: **Het voorkomen of verminderen van ontsteking van de darmwand veroorzaakt door chemotherapie.**
4. Type aanvraag:
   - ☐ **nieuwe aanvraag projectvergunning**
5. Contactgegevens DEC:
   - naam: DEC-RUG
   - telefoonnummer contactpersoon: [redacted] /
     [redacted]
   - mailadres contactpersoon: [redacted]
6. Adviestraject (data dd-mm-jjjj):
   - ☐ ontvangen door DEC: **11-08-2015**
   - ☐ aanvraag compleet: **11-08-2015**
   - ☐ in vergadering besproken: **20-08-2015, 15-10-2015**
   - ☐ anderszins behandeld: **16-10-2015, 02-11-2015, 10-11-2015, 18-11-2015**
   - ☐ termijnonderbreking(en) van / tot: **27-08-2015 tot 08-10-2015, 10-10-2015 tot 29-10-2015, 02-11-2015 tot 03-11-2015, 10-11-2015 tot 13-11-2015, 18-11-2015 tot 18-11-2015 (1 dag)**
   - ☐ besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen **n.v.t.**
   - ☐ aanpassing aanvraag: **08-10-2015, 29-10-2015**
   - ☐ advies aan CCD: **24-11-2015**
7. Eventueel horen van aanvrager **n.v.t.**

- Datum
- 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: **16-10-2015, 02-11-2015, 10-11-2015, 18-11-2015**

Strekking van de vraag / vragen

Vragen t.a.v. Bijlage 1:
U wilt in dit projectvoorstel gebruik maken van een gevalideerd mucositismodel bij de rat geïnduceerd door (éénmalige?) methotrexaat-toediening. Daarnaast noemt U nog “irinotecan or other chemotherapeutics” als optioneel voor modelinductie. Waar hangt de toepassing van farmaca anders dan methotrexaat van af of is dit van toepassing op een beoogd muismodel?

Kunt u specifieke go/no go-indicaties aangeven voor eventueel gebruik van irinotecan or other chemotherapeutics. Overweegt u, bij eventuele toepassing daarvan, hiervoor een nieuwe aanvraag in te dienen?

Volstaat éénmalige methotrexaat-toediening in principe ter inductie van mucositis? U schrijft “During some experiments animals might receive multiple cycles of chemotherapy to investigate the effect of previous cycles on the outcome measures”. Kunt U dit nader toelichten en aan hoeveel cycli wordt gedacht bij de rat? Kunt U ook het bijpassend (cumulatief) ongerief inschatten?

U geeft aan dat “whenever possible parameters which do not require the animals to be sacrificed are chosen.” Aan welke uitleesparameters wordt gedacht met het oog op de statistische onderbouwing? Kennelijk verwacht U dat niet alle dieren geëuthanaseerd hoeven te worden, nietwaar?

Vragen t.a.v. Bijlage 2:

U schrijft “During some experiments animals might receive multiple cycles of chemotherapy to investigate the effect of previous cycles on the outcome measures”. Kunt U dit nader toelichten en aan hoeveel cycli wordt gedacht bij de muis? Kunt U ook het bijpassend (cumulatief) ongerief inschatten?

U geeft aan dat “whenever possible parameters which do not require the animals to be sacrificed are chosen.” Aan welke uitleesparameters wordt gedacht met het oog op de statistische onderbouwing? Kennelijk verwacht U dat niet alle dieren geëuthanaseerd hoeven te worden, nietwaar?
-

- Datum antwoord: **29-10-2015, 03-11-2015, 13-11-2015, 18-11-2015**

- Strekking van het (de) antwoord(en) **De gevraagde verduidelijkingen zijn verwerkt in het projectvoorstel en de bijlages. De antwoorden hebben geleid tot aanpassing van de aanvraag.**

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 (dierproeven in de zin der wet) **Ja.**
2. De aanvraag betreft een nieuwe aanvraag. **Ja.**
3. De DEC is competent om hierover te adviseren. **Ja.**
4. Vanwege betrokkenheid bij het betreffende project is een aantal DEC-leden, met het oog op onafhankelijkheid en onpartijdigheid, niet betrokken bij de advisering. **NVT.**

C. Beoordeling (inhoud):

1. Het project is:

   - **uit wetenschappelijk oogpunt verantwoord**

2. De in de aanvraag aangekruiste doelcategorie(ën) is / zijn in overeenstemming met de hoofddoelstelling(en). **Ja.**
3. De DEC onderschrijft het belang van de doelstelling. Het wordt ingeschat als **reëel**.
4. De gekozen strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling binnen het kader van het project. **Ja.**
5. Er is sprake van de volgende bijzonderheden op het gebied van categorieën 11, 13 en 13c3 van dieren, omstandigheden of behandeling van de dieren. De keuze hiervoor is voldoende wetenschappelijk onderbouwd.

6. Het ongerief als gevolg van de dierproeven is realistisch ingeschat en geclassificeerd. Herhaalde methotrexaat toediening nog onderbouwen.
7. Er zijn vooralsnog geen methoden die de voorgestelde dierproeven geheel of gedeeltelijk zouden kunnen **vervangen**.
8. In het project wordt optimaal tegemoet gekomen aan de vereiste van de **vermindering** van dierproeven daar het meten van citrulline in het bloed herhaalde meting aan hetzelfde dier mogelijk maakt. Het maximale aantal te gebruiken dieren lijkt realistisch ingeschat. De aanvrager beschikt over voldoende expertise en informatie om, bij dit wettelijk vereiste onderzoek, te voorkomen dat onnodige duplicatie plaatsvindt.
9. Het project is in overeenstemming met de vereiste van de **verfijning** van dierproeven en het project is zo opgezet dat de dierproeven zo humaan mogelijk kunnen worden uitgevoerd ondermeer gezien de toepassing van anesthesie bij methotrexaat gavage. Er is geen sprake van belangwekkende milieueffecten.
10. De niet-technische samenvatting is een evenwichtige weergave van het project en begrijpelijk geformuleerd. **Ja**.

D. Ethische afweging

De DEC ziet dit project als een toetsbare eenheid. Het is een voorbeeld van een project dat in de concept CCD-notitie wordt beschreven onder voorbeeld 4: verschillende parallel uit te voeren deelprojecten die alle bijdragen aan het bereiken van het hoofddoel, in dit project het ontwikkelen van een (preventieve) therapie tegen mucositis. Dit is een frequent voorkomende complicatie, die bij ongeveer 55% van de kinderen, die chemotherapie ondergaan, optreedt en er is nog geen specifieke (preventieve) therapie tegen deze complicatie. Het onderhavige onderzoek heeft als doel de mogelijkheden van (preventieve) therapie van mucositis te vergroten en wel via drie pijlers, te weten de voeding, de samenstelling der darmwand en het ‘microbioom’ van de darm. De voor een ethische toetsing centrale vraag of dit directe doel en het hierboven genoemde uiteindelijk doel het uit te voeren onderzoek rechtvaardigen wordt door de DEC positief beantwoord. Hiertoe hanteren wij de volgende overwegingen:

De voorgestelde dierproeven dragen allen bij tot het bereiken van het hoofddoel van de aanvraag, namelijk te komen tot optimalisatie van de (preventieve) therapie van mucositis. Zoals betoogd vormt het geheel daarbij een goed toetsbare eenheid.

Per dierproef zijn de aantallen benodigde dieren inzichtelijk ingeschat met voldoende aandacht voor vermindering en verfijning. De onderzoeksgroep is bij uitstek gekwalificeerd voor het uitvoeren van dit onderzoek en beschikt over voldoende expertise om te voorkomen dat onnodige duplicatie plaatsvindt.

Ook het beschreven ongerief bij de voor het onderzoek te gebruiken ratten en muizen acht de DEC gerechtvaardigd gegeven de doeleinden van het project. Het is uit wetenschappelijk oogpunt verantwoord en het is waarschijnlijk dat de doeleinden worden gehaald. Op termijn kan het project mogelijk voordelen opleveren voor een betere chemotherapeutische behandeling bij de mens en daarmee hopelijk soelaas bieden voor patiënten, die vooralsnog onbehandelbaar zijn voor mucositis als complicatie.

Op grond van alle voor de afweging relevante argumenten komt de DEC-RuG tot de conclusie dat dit onderzoek ethisch toelaatbaar en toetsbaar is, derhalve adviseert de DEC-RuG tot vergunningverlening.

E. Advies

1. Advies aan de CCD

De DEC adviseert de vergunning te verlenen onder de volgende voorwaarde:

-Dat in onderhavige aanvraag alleen methotrexaat als mucositis inducerend chemotherapeuticum wordt gebruikt-

2. Het uitgebrachte advies is gebaseerd op consensus.

7

Centrale Commissie Dierproeven

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

Onze referentie
Aanvraagnummer
AVD105002015338

Bijlagen
2

Datum 1 december 2015
Betreft Ontvangstbevestiging Aanvraag projectvergunning Dierproeven

Geachte [redacted]
Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 27 november 2015.
Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD105002015338. 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:	10500
Naam instelling of organisatie:	Rijksuniversiteit Groningen
Naam portefeuillehouder of diens gemachtigde:	[redacted]
KvK-nummer:	1179037
Straat en huisnummer:	A. Deusinglaan 1, [redacted]
Postcode en plaats:	9713 AV GRONINGEN
IBAN:	NL80ABNA0446049352
Tenaamstelling van het rekeningnummer:	Rijksuniversiteit Groningen

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

Gegevens plaatsvervangende verantwoordelijke onderzoeker

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

Over uw aanvraag

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

Over uw project

Geplande startdatum:	1 december 2015
Geplande einddatum:	1 december 2020
Titel project:	To change the course of chemotherapy-induced mucositis
Titel niet-technische samenvatting:	Het voorkomen of verminderen van ontsteking aan de darmwand veroorzaakt door chemotherapie
Naam DEC:	DEC-RUG
Postadres DEC:	A. Deusinglaan 1,[redacted]
E-mailadres DEC:	secrdec.umcg@umcg.nl

Betaalgegevens

De leges bedragen:	€ 741,-
De leges voldoet u:	na ontvangst van de factuur

Checklist bijlagen

Er zijn geen bijlagen ontvangen.

Ondertekening

Naam:	[redacted]
Functie:	[redacted]
Plaats:	Groningen
Datum:	25 november 2015

Centrale Commissie Dierproeven

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Onze referentie
Aanvraagnummer
AVD105002015338

Bijlagen
2

Datum 1 december 2015
Betreft Factuur aanvraag projectvergunning Dierproeven

Factuur
Factuurdatum: 1 december 2015
Vervaldatum: 31 december 2015
Factuurnummer: 15700338

Omschrijving	**Bedrag**
Betaling leges projectvergunning dierproeven Betreft aanvraag AVD105002015338	€ 741,00

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.

8

[redacted]

Van: [redacted]
Verzonden: maandag 21 december 2015 10:43
Aan: Info-zbo
CC: Secretariaat DEC
Onderwerp: RE: toelichting bij advies AVD105002015338 (RUG code 8024)
Bijlagen: Vragen CCD naar DEC aanvraag [redacted] 181215.docx

Categorieën: Dossier: [redacted]

Beste [redacted]

Hierbij, voor zover mogelijk, antwoorden op uw vragen. Zie attachment.

Vr. gr.
[redacted] EC-RuG

From: Info-zbo [mailto:info@zbo-ccd.nl]
Sent: donderdag 17 december 2015 15:24
To: [redacted]
Subject: toelichting bij advies AVD105002015338 (RUG code 8024)

Geachte leden van DEC Groningen,

De CCD heeft een aanvraag voor projectvergunning ontvangen waarover uw DEC advies heeft uitgebracht. Het betreft het project: 'To change the course of chemotherapy-induced mucositis" met aanvraagnummer AVD105002015338, interne RUG code 8024. Wij willen u om toelichting vragen over enkele onduidelijkheden in de aanvraag en mogelijk is dit besproken in de vergadering van de DEC.

Bijlage 1: U heeft gesproken met de aanvrager over het gebruik van methodextraat en eventuele andere chemotherapeutica. In uw advies stelt u als voorwaarde enkel het gebruik van methodextraat als chemotherapeuticum voor deze projectaanvraag.
In de bijlage welke wij hebben ontvangen staan de overige farmaca of chemotherapeutica nog wel genoemd. Kunt u het antwoord van de onderzoeker op de betreffende vragen meer duiden? Is het mogelijk om het beschreven model op te zetten met alleen methodextraat? Geldt dit dan ook voor bijlage 2 waarin ditzelfde model opgezet wordt in muizen, of kan op basis van de uitkomsten in ratten, bijlage 1, er een noodzaak ontstaan andere chemotherapeutica in het muismodel te testen?

De aanvrager beschrijft dat de ratten individueel worden gehuisvest; "The animals will be housed individually. This is needed since group housing increases stress and in the model the increased stress leads to worsening of the symptoms. In turn this also leads to more variability between rats". De aanvrager voert als argument aan dat individueel huisvesten ook wenselijk is omdat de dieren een canule hebben en aan een swiffel systeem zijn gekoppeld.
Heeft de DEC dit besproken? Het individueel huisvesten is vanuit experimentele handelingen volledig te onderbouwen, maar kan bij ratten niet aangenomen worden dat het individueel huisvesten meer stress veroorzaakt dan groepshuisvesting en vanuit deze redenatie juist rekening gehouden moet worden met stress effecten op experimentele uitkomsten door het individueel huisvesten?
In bijlage 2 wordt voor mannelijke muizen dezelfde redenatie gevolgd, en deze zou voor het individueel huisvesten van mannelijke muizen wel meer navolgbaar zijn.

Heeft u in uw vergadering gesproken over de tijdsplanning van de uitvoer van bijlage 2, het muis model? De aanvrager geeft aan dat dit afhankelijk is van uitkomsten uit bijlage 1. Vind u dat hier duidelijke keuzemomenten gedefinieerd moeten zijn?

Wij willen aan de aanvrager nog de volgende vragen voorleggen:
In bijlage 1 en 2 wordt bij het cumulatief ongerief gerefereerd aan DEXA of MRI scans. Dit is niet beschreven in de tekst van de bijlagen, dit moet nader beschreven worden.

In bijlage 1 beschrijft u het gebruik van antibiotica, naast pre/pro- en synbiotica. In bijlage 2 beschrijft u alleen het gebruik van pre/pro- en synbiotica, is dit een bewuste keuze of een tekstuele verschrijving?

In afwachting van uw antwoord met vriendelijke groet,

[redacted]

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E: info@zbo-ccd.nl (let op: nieuw emailadres!)

Bijlage 1: U heeft gesproken met de aanvrager over het gebruik van methodextraat en eventuele andere chemotherapeutica. In uw advies stelt u als voorwaarde enkel het gebruik van methodextraat als chemotherapeuticum voor deze projectaanvraag.

In de bijlage welke wij hebben ontvangen staan de overige farmaca of chemotherapeutica nog wel genoemd. Kunt u het antwoord van de onderzoeker op de betreffende vragen meer duiden?

De overige farmaca/chemotherapeutica staan er nog ten onrechte. U zou de aanvrager kunnen vragen dit te verwijderen.

De vraag die door de DEC gesteld is (staat tevens in adviesformulier): 'Kunt u specifieke go/no go-indicaties aangeven voor eventueel gebruik van irinotecan or other chemotherapeutics. Overweegt u, bij eventuele toepassing daarvan, hiervoor een nieuwe aanvraag in te dienen?'

Antwoord onderzoeker: 'Irinotecan en andere chemotherapeutica willen we gaan gebruiken in het model wanneer er succesvolle interventies zijn gevonden bij MTX. Het klopt inderdaad dat het werkingsmechanisme van de chemotherapeutica anders is, maar het ziektebeeld, de mucositis, is vergelijkbaar tussen de verschillende chemotherapeutica. Het doel van de chemotherapeutica is om de celdeling te stoppen en dit doen ze op een verschillende manier, de uitkomst is alleen wel vergelijkbaar. Interventies die we vinden bij MTX werken mogelijk ook bij andere chemotherapeutica, alleen dit zal eerst onderzocht moeten worden. Het doel van dit project is om het verloop van chemotherapie-geïnduceerde mucositis te veranderen, volgens ons past het onderzoeken van de effectiviteit van succesvolle interventies bij een ander chemotherapeuticum binnen dit doel'

Naar oordeel van de DEC is onderzoek met een ander chemotherapeuticum met een ander werkingsmechanisme, zoals irinotecan, een afwijkend onderzoeksdoel welke beter ook omschreven zou moeten worden. Er zijn ook geen go/no go criteria voor aangegeven. In tegenstelling tot methotrexaat, is mucositis-inductie middels irinotecan een nog niet voldoende ontwikkeld model. Bij toepassing hiervan zou een nieuwe aanvraag passen

Is het mogelijk om het beschreven model op te zetten met alleen methodextraat?

Ja, middels een ruim aantal referenties genoemd in de aanvraag blijkt dit. Het is een gevalideerd model en wordt ook als zodanig door aanvrager benoemd. De DEC is bekend met het eerdere (methotrexaat-geinduceerde) mucositis werk van de aanvrager en heeft in het advies benoemd dat de onderzoeksgroep bij uitstek gekwalificeerd is voor het type werk.

Geldt dit dan ook voor bijlage 2 waarin ditzelfde model opgezet wordt in muizen

Ja

, of kan op basis van de uitkomsten in ratten, bijlage 1, er een noodzaak ontstaan andere chemotherapeutica in het muismodel te testen?

Dat kan niet. De onderzoeksvraag in deze aanvraag is of de interventie (bestaande uit een aantal verschillende invalshoeken), (effecten van) mucositis kan verminderen. Het methotrexaat model is gevalideerd en is de basis voor het testen van de interventie.

Hieruit zal geen ‘noodzaak voor het testen van andere chemotherapeutica in muizen kunnen ontstaan’.

De aanvrager beschrijft dat de ratten individueel worden gehuisvest; “The animals will be housed individually. This is needed since group housing increases stress and in the model the increased stress leads to worsening of the symptoms. In turn this also leads to more variability between rats”. De aanvrager voert als argument aan dat individueel huisvesten ook wenselijk is omdat de dieren een canule hebben en aan een swiffel systeem zijn gekoppeld.

Heeft de DEC dit besproken? Het individueel huisvesten is vanuit experimentele handelingen volledig te onderbouwen, maar kan bij ratten niet aangenomen worden dat het individueel huisvesten meer stress veroorzaakt dan groepshuisvesting en vanuit deze redenatie juist rekening gehouden moet worden met stress effecten op experimentele uitkomsten door het individueel huisvesten?

In bijlage 2 wordt voor mannelijke muizen dezelfde redenatie gevolgd, en deze zou voor het individueel huisvesten van mannelijke muizen wel meer navolgbaar zijn.

Dit is niet door de DEC besproken. De aanvrager meldt dat stress een factor is waar rekening mee wordt gehouden t.a.v. de gegeven dosis methotrexaat in ratten.

Heeft u in uw vergadering gesproken over de tijdsplanning van de uitvoer van bijlage 2, het muis model? De aanvrager geeft aan dat dit afhankelijk is van uitkomsten uit bijlage 1. Vind u dat hier duidelijke keuzemomenten gedefinieerd moeten zijn?

Er is niet gesproken over tijdsplanning van de uitvoer van bijlage 2. Uw vraag over de keuzemomenten vraagt naar een nieuw te vormen mening van de DEC t.a.v. dit punt. Die is op deze korte termijn (een aantal dagen responsie tijd) niet te bepalen aangezien er geen vergadering is.

Wij willen aan de aanvrager nog de volgende vragen voorleggen:

In bijlage 1 en 2 wordt bij het cumulatief ongerief gerefereerd aan DEXA of MRI scans. Dit is niet beschreven in de tekst van de bijlagen, dit moet nader beschreven worden.

In bijlage 1 beschrijft u het gebruik van antibiotica, naast pre/pro- en synbiotica. In bijlage 2 beschrijft u alleen het gebruik van pre/pro- en synbiotica, is dit een bewuste keuze of een tekstuele verschrijving?

10

[redacted]

Van: [redacted]
Verzonden: dinsdag 22 december 2015 10:08
Aan: Info-zbo
Onderwerp: RE: Dossier AVD105002015338
Bijlagen: 8024-1 [redacted] NTS (1).docx; 20151221 Bijlage 1 projectvoorstel mucositis.docx; 20151221 Bijlage 2 projectvoorstel mucositis.docx; 20151221 Projectvoorstel mucositis (1).docx; Aanvraag project mucositis (1).docx

Categorieën: Dossier [redacted]

Geachte mevrouw [redacted]

Naar aanleiding van de vragen van de CCD hebben wij een aantal aanpassingen gedaan in de tekst. Hieronder de reactie op elk van de vragen.

In bijage dierproeven 3.4.4.1 en 3.4.4.2 refereert u bij het cumulatief ongerief aan DEXA of MRI scan. Deze handeling wordt niet verder uitgewerkt in de tekst.
In bijlage 3.4.4.1 en 3.4.4.2 (2.A.1) is de tekst aangepast zodat de handelingen uitgewerkt worden.

“With a DEXA-scan or nuclear magnetic resonance spectroscopy we can measure the composition of the body, this shows in which compartment the animals lose weight (fat tissue, muscle or combination). Feeding strategies could be designed to target the loss of weight in a certain compartment and try to prevent it.”

In bijlage 3.4.4.1 beschrijft u het gebruik van antibiotica, naast pre/pro- en synbiotica. In bijlage 3.4.4.2 beschrijft u alleen het gebruik van pre/pro- en synbiotica, is dit een bewuste keuze of een tekstuele verschrijving?
Dit was een tekstuele verschrijving en is aangepast zodat bij beide antibiotica staat.

U beschrijft dat de uitvoer en keuzes die gemaakt worden voor het uitvoeren/ opzetten van dit model in de muis (bijlage 3.4.4.2), mogelijk afhankelijk zijn van uitkomsten uit bijlage 3.4.4.1 Kunt u de keuzemomenten en eventuele go/no go momenten en de tijdsplanning van bijlage 3.4.4.1 ten opzichte van bijlage 3.4.4.2 meer uitwerken in de tekst?
Om het verloop en go/no go momenten te verduidelijken in de tekst is de tekst in het projectvoorstel aangepast bij onderdeel 3.4.2 (de laatste paragraaf)

“The chemotherapy-induced mucositis mouse model will assess mechanisms of successful strategies which require knock-outs or other tests currently unavailable in rats. The mouse-model will be developed independent of the results of earlier studies, in year two of the project. Once established, it will only be used to study interventions which seem successful in the rat model, but which need knock-outs or other tests currently unavailable in rats. “

De DEC heeft als voorwaarde gesteld dat dit project alleen met methotrexaat wordt uitgevoerd, en hier ook met u over gecorrespondeerd. Zou u uit de tekst van de bijlagen de andere farmaca en chemotherapeutica kunnen verwijderen om onduidelijkheid te voorkomen?
Dit is aangepast in de tekst zoals u voorstelt.

In bijlage 3.4.4.1 beschrijft u het individueel huisvesten van ratten. De keuze voor individueel huisvesten van de dieren is vanuit de experimentele opzet en –handelingen te rechtvaardigen, maar uw redenatie

dat ratten bij individuele huisvesting minder stress ervaren dan bij groepshuisvesting is niet algemeen geaccepteerd. Kunt u deze tekst heroverwegen.
We hebben de tekst aangepast en het statement over individueel huisvesten en stress eruit gehaald om verwarring te voorkomen.

Hopelijk hebben wij de verschillende vragen van de CCD kunnen oplossen. Mochten er nog aanvullende vragen zijn horen wij het graag.

Met vriendelijke groet,
[redacted]

Van: Info-zbo [info@zbo-ccd.nl]
Verzonden: maandag 21 december 2015 11:34
Aan: [redacted]
CC: [redacted]
Onderwerp: Dossier AVD105002015338

Geachte [redacted],

U heeft een aanvraag tot projectvergunning gedaan bij de CCD. het betreft dossier AVD105002015338 getiteld: 'To change the course of chemotherapy-induced mucositis". In uw aanvraag zitten nog een aantal onduidelijkheden. Wij hebben ook aan de DEC om nadere toelichting gevraagd.

Het antwoord op de vragen zal naar onze mening leiden tot tekstuele aanpassing van de bijlagen 3.4.4.1 en 3.4.4.2

In bijage dierproeven 3.4.4.1 en 3.4.4.2 refereert u bij het cumulatief ongerief aan DEXA of MRI scan. Deze handeling wordt niet verder uitgewerkt in de tekst.

In bijlage 3.4.4.1 beschrijft u het gebruik van antibiotica, naast pre/pro- en synbiotica. In bijlage 3.4.4.2 beschrijft u alleen het gebruik van pre/pro- en synbiotica, is dit een bewuste keuze of een tekstuele verschrijving?

U beschrijft dat de uitvoer en keuzes die gemaakt worden voor het uitvoeren/ opzetten van dit model in de muis (bijlage 3.4.4.2), mogelijk afhankelijk zijn van uitkomsten uit bijlage 3.4.4.1 Kunt u de keuzemomenten en eventuele go/no go momenten en de tijdsplanning van bijlage 3.4.4.1 ten opzichte van bijlage 3.4.4.2 meer uitwerken in de tekst?

De DEC heeft als voorwaarde gesteld dat dit project alleen met methotrexaat wordt uitgevoerd, en hier ook met u over gecorrespondeerd. Zou u uit de tekst van de bijlagen de andere farmaca en chemotherapeutica kunnen verwijderen om onduidelijkheid te voorkomen?

In bijlage 3.4.4.1 beschrijft u het individueel huisvesten van ratten. De keuze voor individueel huisvesten van de dieren is vanuit de experimentele opzet en –handelingen te rechtvaardigen, maar uw redenatie dat ratten bij individuele huisvesting minder stress ervaren dan bij groepshuisvesting is niet algemeen geaccepteerd. Kunt u deze tekst heroverwegen.

In verband met de planning en de behandeling in de CCD vergadering zou het prettig zijn als u de antwoorden/ aangepaste bijlagen woendag 23 december 2015 aan ons verstuurd worden,

Met vriendelijke groet, [redacted]

Centrale Commissie Dierproeven
www.centralecommissiedierproeven.nl
……

Postbus 20401 | 2500 EK | Den Haag
……
T: 0900 2800028
E: info@zbo-ccd.nl (let op: nieuw emailadres!)

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'.	10500
1.2	Provide the name of the licenced establishment.	Rijksuniversiteit Groningen
1.3	Provide the title of the project.	To change the course of chemotherapy-induced mucositis

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.

Survival of children with cancer has increased significantly over the last decades, due to more intensive treatment protocols. The disadvantage of these more intensive chemotherapy treatment protocols is a higher frequency of side effects. One of these side effects is gastrointestinal mucositis (hereafter mucositis); a complex inflammatory reaction of the mucous membranes of the alimentary tract. Mucositis develops via different biological stages resulting in villus atrophy, ulceration and loss of barrier function, ending with spontaneous healing. The patients suffer from vomiting, abdominal pain, diarrhea, weight loss and are at increased risk of developing a bacteremia or sepsis. Mucositis leads to a lower quality of life and an increased morbidity and mortality. So far no interventions are found which prevents or to treat GI mucositis.

In this project we aim to change to course of chemotherapy-induced mucositis. To be able to achieve the aim, both fundamental and translational research is required.

Mucositis is a complex multifactorial process with many different established contributors (intestinal epithelium, immune system and nutrition). Based on these contributors a 5-phase model was developed for the pathobiology of oral mucositis.[1] This model is also the basis for the pathobiology of gastrointestinal mucositis. However, as one can imagine the environment in the intestine is markedly different from the oral environment, and thus the pathophysiology of gastrointestinal mucositis is probably (slightly) different. In short, the 5-phase model describes an initiation phase during which nuclear factor kappa B (NFκB) is activated by the formation of reactive oxygen species (ROS) and the occurrence of damage to the DNA. This is followed by the primary damage response phase during which messenger molecules / cytokines (among others: tumour necrosis factor α (TNFα)) are induced which lead to tissue inflammation and apoptosis. In the amplification phase the messenger molecules / cytokines are amplified due to positive feedback loops leading to increased inflammation and apoptosis. During the ulcerative phase the epithelial barrier integrity is lost due to the apoptosis and intestinal damage, which is spontaneously healed during the healing phase. However, in the gut there are other possible contributors which need to be explored for example the gut microbiota.[2,3] These microbiota have been reported to play a role in gastrointestinal mucositis in several studies so far.

In this project we will do fundamental research into a possible contributor which is not included in the 5-phase model, the gut microbiota.[2] The gut is in a continuous state of low grade infection due to the interactions between the gut microbiota and the immune system. Previous research has shown there is a change in microbial composition of the microbiota after chemotherapy, coinciding with gastrointestinal mucositis.[3] These changes in the microbiota potentially influence the inflammatory reaction seen during mucositis, since a change in composition of the gut microbiota would also alter the balance in the continuous state of low grade infection in the intestine. Interventions aimed to influence the composition of the gut microbiota could positively influence the course of mucositis by promoting a healthy composition of the microbiota, which could restore the normal state of low-grade inflammation in the intestine.

The second component we want to investigate in this project are interventions aimed to influence the inflammatory response and/or improve the intestinal barrier. The 5-phase model is mainly revolved around the inflammatory reaction present during mucositis. Drugs which influence this inflammatory reaction can potentially influence the course of gastrointestinal mucositis by reducing the damage done by the inflammation. An integral part of the damage done during mucositis is the ulceration phase, during which the patient suffers from the loss of the barrier function. Therapies which improve the barrier function by for example promoting proliferation, reducing apoptosis, improving cell junctions and/or preventing cellular changes, could reduce or prevent the damage to the intestinal barrier which is the main problem for the patient.
The last component we will investigate are nutritional based interventions. In previous research performed by our group the absorption of different components of the nutrition during mucositis were investigated.[4] Nutrition is known to be able to influence the barrier function of the intestine during mucositis and other intestinal complications. In this study we will continue investigations into potential nutritional interventions which improve the barrier function and could help speed up the recovery after the ulceration phase.
A strength of this study is the potential of the different components of the study to influence each other. The nutrition is for example known to influence the composition of the microbiota, which could lead to double beneficial effects.

The model we developed and will use in this project is well validated and suitable to test interventions based on the results of our basic research. Therefore, during this project translational research will be performed to study whether we can change the course of chemotherapy-induced mucositis.

(1) [redacted]
(2) [redacted].
(3) [redacted]
[redacted]
(4) [redacted]
[redacted]

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 find prevention or treatment strategies which change the course of severe mucositis. To achieve this objective several potential strategies will be investigated namely, the gut microbiota, therapies/prophylaxis and nutrition. Also the mechanism behind the interventions will be investigated.

We deem the aim of this project feasible within the duration of this project. In previous projects the chemotherapy-induced mucositis rat model which we will use in this project was developed. The model is now well validated and there is a tremendous amount of experience with the model.[1-4] The project consists of different research avenues which will be investigated simultaneously with good communication between the different contributors. The combination of the different areas of research and combination of knowledge gained within each area will help us achieve the aim of the project.

3.3 Relevance

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

Previous research has shown that 55% of the children receiving chemotherapy develop intestinal mucositis. On top of that when patients were asked the most debilitating side-effect of chemotherapy, symptoms of mucositis were mentioned the most. The overall consequences of the mucositis not only involves quality of life after chemotherapy courses, but probably also decreases survival due to treatment reduction, treatment delay, decreased nutritional state, which on its own is associated with treatment complications and increased mortality. Up until now there are no interventions in place which prevent or improve the course of mucositis, which we want to change with this project.

(1) [redacted]

(2) [redacted]

(3) [redacted]

(4) [redacted]

3.4 Research strategy

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

This projects aims to change the course of chemotherapy-induced mucositis and their mechanism of action. Different potential prevention or treatment strategies will be assessed in a chemotherapy-induced mucositis rat model.

The first strategy is to investigate the relevance of intervening at the level of the gut microbiota. The pathophysiology of the possible relationship between the GI microbiota and mucositis has been extensively reviewed in a paper of our research group. However, little research to investigate the relationship has been done until this point. We investigated the microbiota of pediatric acute myeloid leukemia (AML) patients, which demonstrated a significant decrease in the total number and a change in composition of the fecal bacteria. This result was replicated in a rat model of chemotherapy-induced mucositis by our group and by others. The relationship between the microbiota and the development and recovery of mucositis and possible interventions is the first possible strategy investigated here. Possible interventions for the microbiota are among others antibiotics to limited potential pathogenic bacteria, pro- and prebiotics to promote a 'healthy' population of the microbiota or a combination of the different interventions.

Secondly, interventions aimed to influence the inflammatory response and/or improve the intestinal barrier will be investigated. Previous research has shown the beneficial effects of inhibition of several cytokines (among others TNFα and IL-1β).[1] Here, we will continue to investigate drugs which influence the inflammatory response during mucositis and further elucidate important components of the inflammatory response during mucositis. [redacted] [2] Another important feature in the previously mentioned 5-phase model is the disruption of the intestinal barrier during the ulceration phase. Interventions aimed to improve the intestinal barrier will be investigated in this study. One approach to improve the barrier function is to limit cell death, this can be accomplished to reduce apoptosis or via increased proliferation of the intestinal epithelial cells. Another approach is to improve the barrier function by interventions aimed at the cellular junctions. Increasing the amount or strength of the cellular junctions (for example tight junctions) between the epithelial cells, the disruption of the intestinal barrier might be prevented or limited. [redacted].[3]

A third possible strategy are interventions based on the nutrition. Previous research in our group has focused on feeding strategies based on parental nutrition or tube feeding strategies and the uptake of nutrients. In this study we will continue investigating optimal feeding strategies, on top of that we will also investigate nutritional interventions focused on supplementation of the intake. [redacted]

The three previously mentioned strategies are closely related, as the interventions investigated could also have an effect on other components. Nutrition is known to influence the microbiota composition in the gut and there is a link between the microbiota and the inflammatory response present, as the intestine is in a constant state of low grade inflammation due to interactions between microbiota and immune system. Therefore, combinations of effective interventions could be investigated to show whether the combination has an additive or synergistic effect on the course of severe mucositis.

To investigate the mechanism behind successful strategies we would like to have knock-outs available. The availability of knock-out rats is far from complete compared to mice. Therefore, we would like to transpose our chemotherapy-induced mucositis rat model into a mouse model to be able to investigate proposed mechanisms for the interventions. As described above, the interaction between the gut microbiota and the immune system is important for the development of mucositis. Therefore, we will study knock out models of genes encoding for receptors which are important for the interaction of this interaction [redacted]). On the other hand the inflammatory response could be hypothesized to be the major contributor to the development of mucositis and a knock-out of a central gene ([redacted]) could reduce the severity of the mucositis. More genes of interest will be decided during the course of the study, depending on new literature and results of above mentioned studies.

The three different research strategies will all be started simultaneously and continue over the course of the entire project. The exact planning of these experiments is hard to predict since the outcomes of earlier experiments taken into account in the development and planning of new experiments. The development of the mouse model will most likely start in year two.

(1) [redacted]
(2) [redacted]
(3) [redacted]

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

The different components of the project will all employ the rat model of chemotherapy-induced mucositis which is a well validated model that has been developed and further employed in previous projects.[1-4] The last part of the project which tries to elucidate the mechanisms of different interventions will be performed in both our established rat model and a mouse model which is to be developed. The mouse model will be developed to have knock-outs available since in rats the knock-outs are limited.

For the first component of the study the chemotherapy-induced rat model of mucositis will be employed during which the relationship between the mucositis and the gut microbiota will be elucidated. To investigate this relationship different experiments will be performed during which (part of) the microbiota is depleted using antibiotics, bacterial strains will be added using probiotics, prebiotics will be given to modulated the composition of the gut microbiota or the effect of the gut microbiota will be mimicked.

The second component aims to prevent or elevate the mucositis by improving of cell survival, limiting inflammation or via other mechanisms. During these experiments the animals will be (prophylactically) treated with drugs which for example could improve cell survival, promote cell proliferations, limit apoptosis or limit inflammation.

The third component aims to intervene in the nutrition. [redacted]

The chemotherapy-induced mucositis mouse model will assess mechanisms of successful strategies which require knock-outs or other tests currently unavailable in rats. The mouse-model will be developed independent of the results of earlier studies, in year two of the project. Once established, it will only be used to study interventions which seem successful in the rat model, but which need knock-outs or other tests currently unavailable in rats.

(1) [redacted]
(2) [redacted]
(3) [redacted]
(4) [redacted]

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

The components all have the goal to change the course of severe mucositis and the interventions are designed to target different factors which could influence the course of mucositis. However, the influence of each of the component is not limited to the component itself. Changes in nutrition also have an influence on the composition of the microbiota and could possibly also improve cell survival. The composition of the microbiota is also known to influence the inflammation in the intestine. Therefore, the different components of the project are closely linked together.

Interventions which improve the course of chemotherapy-induced mucositis will be selected and interventions combined if possible to investigate possible synergistic effects.

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	Chemotherapy-induced mucositis rat model
2	Chemotherapy-induced mucositis mouse model
3	
4	
5	
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'.	10500
1.2	Provide the name of the licenced establishment.	Rijksuniversiteit Groningen
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 1 — Type of animal procedure: Chemotherapy-induced mucositis rat model

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

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

To assess the course of chemotherapy-induced mucositis a rat model was developed previously in our department.[1] To be able to investigate the chemotherapy-induced gastrointestinal mucositis (hereafter mucositis) the rats in our model receive an injection of methotrexate (MTX) after which the mucositis can be investigated. During a bout of mucositis the rats will develop diarrhoea, reduce their food intake and as such reduce in body weight. These parameters will be measured throughout the whole experiment. With a DEXA-scan or nuclear magnetic resonance spectroscopy we can measure the

composition of the body, this shows in which compartment the animals lose weight (fat tissue, muscle or combination). Feeding strategies could be designed to target the loss of weight in a certain compartment and try to prevent it.

When we study different feeding strategies currently used in patients (parental feeding and tube feeding) a rat model with a catheter into the vena jugularis or the duodenum was developed. These rats will receive surgery during which they received a permanent catheter in the duodenum or vena jugularis which will be subcutaneously tunnelled to the head, where attachment to the swivel system is possible. The swivel system is used to continuously infuse parenteral or tube feeding. After the operation which places the catheter the rats receive analgesics for 24 hours and are enabled to recover for seven days. The dose of the MTX in this model is lowered since the animals are under increased stress due to the catheter.

Primary outcome measures:

- Histology of the intestine: To determine the severity of mucositis, the histology of the intestine will be investigated. The histology will be measured on time points of interest when animals are sacrificed.
- Blood citrulline levels: To reduce the amount of animals needed the blood levels of citrulline and/or other (blood) markers will be measured during the experiment. Citrulline is an amino-acid almost exclusively produced by enterocytes, the blood levels of citrulline are a marker for the enterocyte mass in the intestine.
- Gut microbiota: To investigate the gut microbiota, intestinal content will be collected in which the microbiota will be measured. However, this is only possible after animals are sacrificed. Therefore, faeces will be collected to investigate the gut microbiota during the experiment.
- Glucose absorption: Before the rats will be sacrificed they will be fasted overnight and a glucose absorption test will be performed, this test is performed to test the intestinal function.

(1) [redacted]

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

In general the previously mentioned MTX-induced rat model will be employed to investigate the effect of different interventions before and/or after administration of the chemotherapeutic agent.

Intervention studies will be carried out with:

- Anti-, Pro-, pre- or synbiotics. These are interventions aimed to enlarge the bacterial population of the microbiota and/or to change its composition. Antibiotics will be aimed to reduce the populations of potentially pathogenic bacteria, these can be given in the food/drinking water or via oral gavage. Probiotics are one or more bacterial strains which are given via oral gavage, prebiotics are substances ([redacted]) which can be given in the food/drinking water or via oral gavage and synbiotics are a combinations of the two. Depending on the aim of the experiment these will be given once or multiple times during the experiment.
- Drugs aimed to influence the inflammatory response. These drugs will most likely be given via an (intravenous, intraperitoneal or subcutaneous) injection, depending on the optimal route of the intervention, as a prophylaxis or therapy. With these studies we aim to influence the inflammatory response in a beneficial way to limit the damage done during mucositis or to fasten the recovery ([redacted]).
- Drugs targeting the intestinal barrier. Drugs improving the intestinal barrier will also most likely be given via an injection, although certain drugs could potentially be given via oral gavage or in the food/drinking water, given as prophylaxis or therapy. These drugs aim to prevent or limit the disruption of the intestinal barrier during the ulceration phase of mucositis [redacted]

- Nutrition. Nutritional interventions include both feeding strategies and supplementation to food/water. [redacted]
- [redacted]

MTX will be administered under general anaesthesia and the rats are followed during the development and recovery of mucositis. Throughout the whole experiment body weight, intake and the presence of diarrhoea are measured. During the experiment blood samples will be taken daily or every other day to measure blood levels of citrulline and/or other biomarkers. Faecal samples will be collected to investigate the gut microbiota during the experiment. Animals will be sacrificed on points of interest which depend on the aim of the experiment.

During some experiments animals might receive two cycles of chemotherapy to investigate the effect of successful interventions during a second cycle of chemotherapy. The effect of successful intervention during two cycles will be investigated since patients also receive multiple during the treatment of their tumour.

The night before the animals are sacrificed the animals are fasted since this is needed to perform a glucose absorption test to investigate the function of the intestine. For this test the animals receive a bolus of labelled glucose via oral gavage and the blood glucose levels will monitored via repeated collection of blood via the tail vein.

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

Before each experiment a sample size calculation based on a power assessment with historical data from our laboratory (expected effect size and standard deviation of histology, blood citrulline levels, etc) will be performed to determine the group size of the animals. To reduce the amount of animals needed, the course of chemotherapy-induced mucositis will in general be monitored by blood citrulline levels, while histology is performed on a time point of interest.

B. The animals

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

We chose wild-type Wistar male rats as animal model in this project since in preceding projects a chemotherapy-induced rat model was developed. This is a validated model and the model is very suitable for the experiments planned in the present project. The model is optimized for male rats during the development of the model, which means the dose in this model is most likely not optimized for female rats. On top of that, the fact that mucositis is a multifactorial in their development adding another variable (male vs female) into the equation would increase the variation too much.

The rats in our experiments will be purchased from commercial sources both from inside and outside of the EU. The life stage of the animals is post-weaning and young adults depending on the aim of the experiment. We estimate that approximately 8 rats are required for each experimental group in which mucositis is induced. The exact number of rats needed for each experiment will be calculated with a sample size calculation based on historical data from our laboratory and a power assessment. With these group sizes, the estimation that each experiment has 3 groups with 2 time points and the estimation of 3 experiments each year of the five years of this project leads to an estimated number of 720 animals needed.

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.

Replacement: The goal of this project is to ameliorate the course of chemotherapy-induced mucositis. Mucositis is a multifactorial process which involves many different established contributors (intestinal epithelium, immune system and nutrition) which are hard to mimic in vitro. On top of that we will investigate the role of the intestinal microbiota. Therefore, an accurate in vitro model which combines all these factors is impossible to accomplish. The experiments are clearly too invasive to be able to investigate them in the human situation. Therefore, an animal model of chemotherapy -induced mucositis is needed.

Reduction: Experiments during which animals are sacrificed at two or more time points compared to the control group(s) will, when the experimental aim allows this, not be measured on each time point since the control animals do not change during the experiment. Also parameters which do not require the animals to be sacrificed are assessed whenever appropriate, for example citrulline in blood samples and faeces samples.

Refinement: The animal model employed in this project is a well validated model in which the development and recovery of mucositis can both be studied. Anaesthesia and analgesia will be used whenever appropriate to minimize stress and discomfort.

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

The animals are handled often to minimize stress and discomfort during weighing or while being transferred. On top of that, animals are monitored frequently to identify potential discomfort as soon as possible. Anaesthesia and analgesia are given whenever needed. Potential environmental-polluting waste will be collected appropriately.

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.

Several possible interventions are investigated which could potentially ameliorate the course of severe mucositis. The role of microbiota during the development and recovery of chemotherapy-induced mucositis has not yet been extensively investigated. The research that has been done so far showed potential but due to flaws in the experimental design results are not solid enough to allow direct translation to humans. Some studies report nutritional

interventions. However, these studies have so far focused on amino acid supplementation, other nutrients or combinations have hardly been studied.

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.

The animals will be housed individually. This is needed since we want to measure food intake, water intake and some experiments require supplementation to the water/food (for example antibiotics). Also the catheterized animals require individual housing due to the connection to a swivelsystem.

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.

The rats will be anesthetized with Isoflurane/oxygen by or under supervision of experienced employees. Analgesia will be used after the catheterization and whenever needed at other moments.

I. Other aspects compromising the welfare of the animals

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

After administration of the chemotherapeutic agent the rats will have 4 days of lowered food intake, weight loss (<10%), diarrhoea and general sickness. Also in the catheter model there is increased stress on the animals, which potentiates effects of mucositis. Therefore, the dose of chemotherapeutics is lowered in this model.

Explain why these effects may emerge.

The goal of our project is to change to course of chemotherapy-induced mucositis, the symptoms of mucositis are an impaired uptake of nutrients and a general sickness. Due to an impaired uptake of nutrients more water will be attracted to the faeces which results in diarrhoea. Combined the general sickness and diarrhoea lead to weight loss.

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

Chemotherapy will be given at a dose which ensures the mucositis is self-limiting as it is in patients. This dose is optimized to minimize severity and suitable to investigate mucositis. The interventions which are investigated are aimed to prevent occurrence or minimise severity of the mucositis.

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.

When signs of severe complications, like severe weight loss (>15%) and general severe malaise (behavior, fur, hygiene) the rats will be sacrificed due to humane endpoints. After injection with the chemotherapeutic agent the rats will be investigated daily.

Indicate the likely incidence.

<5% of the animals receiving chemotherapy.

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').

Non-recovery: collection of organs after being sacrificed
Mild: individual housing, transport during experiments, oral gavage, collection of blood from tail vein, injection of intervention (IV,IP,SC), dexa-scan or nuclear magnetic resonance spectroscopy, faecal transfer, repeated collection of blood during glucose absorption test, fasting and continuous infusion of parenteral or tube feeding.
Moderate: Administration of chemotherapy/NaCl via injection, experiencing mucositis and placement of permanent catheter into the vena jugularis or duodenum.
The overall expected level of discomfort is moderate. The animals will be closely monitored after administration of chemotherapy until the end of the experiment.

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.

To be able to investigate the histology of the intestine to evaluate the mucositis and its recovery and to collect the content of the gastrointestinal tract to evaluate the gut microbiota. Other organs are also investigated and harvested depending on the research question.

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'.	10500
1.2	Provide the name of the licenced establishment.	Rijksuniversiteit Groningen
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 2 Type of animal procedure: Chemotherapy-induced mucositis mouse model

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.

Since the aim with the mouse model is to replicate the results from the rat model to have knock-outs available, the mouse model will be designed to be as similar as possible to the rat model as possible given the differences between the two species. Therefore, there will be overlap between the outcome measures and interventions possibly performed in both models.

The main reason to develop a methotrexate (MTX)-induced mucositis mouse model is the availability of transgenic mouse models enabling mechanistic studies of causes of mucositis. The mouse model will be developed based on available literature combined with experience we have with the rat model. Based on literature a suitable mouse strain will be selected and an approximate chemotherapy dose will be determined to be given IV and in a single dose. The dose will be optimised in several experiments to ensure minimal severity for the mouse, combined with a suitable model to investigate mucositis.

To be able to investigate the MTX-induced gastrointestinal mucositis (hereafter mucositis) the mice in our model will receive an injection of MTX after which the mucositis can be investigated. During a bout of mucositis the mice will develop diarrhoea, reduce their food intake and as such reduce in body weight. These parameters will be measured throughout the experiments. With a DEXA-scan or nuclear magnetic resonance spectroscopy we can measure the composition of the body, this shows in which compartment the animals lose weight (fat tissue, muscle or combination). Feeding strategies could be designed to target the loss of weight in a certain compartment and try to prevent it.

Primary outcome measures:

- Histology of the intestine: To determine the severity of mucositis, the histology of the intestine will be investigated. The histology will be measured on time points of interest when animals are sacrificed.
- Blood citrulline levels: To reduce the amount of animals needed the blood levels of citrulline and/or other (blood) markers will be measured during the experiment. Citrulline is an amino-acid almost exclusively produced by enterocytes, the blood levels of citrulline are a marker for the enterocyte mass in the intestine.
- Gut microbiota: To investigate the gut microbiota, intestinal content will be collected in which the microbiota will be measured. However, this is only possible after animals are sacrificed. Therefore, faeces will be collected to investigate the gut microbiota during the experiment.
- Glucose absorption: Before the rats will be sacrificed they will be fasted overnight and a glucose absorption test will be performed, this test is performed to test the intestinal function.

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

When the mice model is successfully developed and optimized, the model will be used to investigate the effect of different interventions before and/or after administration of the chemotherapeutic agent.

The main objective in the mouse model will be to confirm improved results found in the rat model and to investigate a genetic involvement in the mechanism of action. Intervention studies will be carried out with:

- Knock-out genes. To investigate the proposed mechanisms of successful interventions an important tool is the use of transgenic mice. The interaction between the gut microbiota and the immune system is important for the development of mucositis. Therefore, we will study knock out models of genes encoding for receptors which are important for the interaction of this interaction ([redacted]). On the other hand the inflammatory response could be hypothesized to be the major contributor to the development of mucositis and a knock-out of a central gene [redacted]) could reduce the severity of the mucositis. More genes of interest will be decided during the course of the study, depending on new literature and results of above mentioned studies.

Since the mouse model will be employed to investigate genetic involvement in the mechanism of action of the interventions which were successful, we will need to confirm whether these interventions are successful in mice as well.

- Anti-, Pro-, pre- or synbiotics. These are interventions aimed to enlarge the bacterial population of the microbiota and/or to change its composition. Probiotics are one or more bacterial strains which are given via oral gavage, prebiotics are substances ([redacted]) which can be given in the nutrition or via oral gavage and synbiotics are a combinations of the two. Depending on the aim of the experiment these will be

given once or multiple times during the experiment.

- Drugs aimed to influence the inflammatory response. These drugs will most likely be given via an (intravenous, intraperitoneal or subcutaneous) injection, depending on the optimal route of the intervention, as a prophylaxis or therapy. With these studies we aim to influence the inflammatory response in a beneficial way to limit the damage done during mucositis or to fasten the recovery [redacted]).
- Drugs targeting the intestinal barrier. Drugs improving the intestinal barrier will also most likely be given via an injection, although certain drugs could potentially be given via oral gavage or in the food/drinking water, given as prophylaxis or therapy. These drugs aim to prevent or limit the disruption of the intestinal barrier during the ulceration phase of mucositis (for example drugs enhancing tight junctions).
- Nutrition. Nutritional interventions are focussed on supplementation of nutrients to food/water. [redacted]
- [redacted]

MTX will be administered under general anaesthesia and the mice are followed during the development and recovery of mucositis. Throughout the whole experiment body weight, intake and the presence of diarrhoea are measured. Blood will be drawn to assess the plasma citrulline levels and faecal samples will be collected to investigate the gut microbiota during the experiment. Animals will be sacrificed on points of interest which depend on the aim of the experiment.

During some experiments animals might receive two cycles of chemotherapy to investigate the effect of successful interventions during a second cycle of chemotherapy. The effect of successful intervention during two cycles will be investigated since patients also receive multiple during the treatment of their tumour.

The night before the animals are sacrificed the animals are fasted since this is needed to perform a glucose absorption test to investigate the function of the intestine. For this test the animals receive a bolus of labelled glucose via oral gavage and the blood glucose levels will monitored via repeated collection of blood via the tail vein.

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

Before each experiment a sample size calculation based on a power assessment with historical data from our laboratory (expected effect size and standard deviation of histology, blood citrulline levels, etc) will be performed to determine the group size of the animals. To reduce the amount of animals needed, the course of chemotherapy-induced mucositis will in general be monitored by blood citrulline levels, while histology is performed on a time point of interest.

B. The animals

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

Both WT and transgenic male mice will be used in this mouse model of chemotherapy-induced mucositis. The strain and life stage of the mice will be determined during the development of the mice model. Male mice will be used to keep rat and mouse model as similar as possible. Since mucositis is a multifactorial in their development adding another variable (male vs female) into the equation would increase the variation too much. The mice will be purchased from commercial sources both from inside and outside of the EU. The life stage of the animals is post-weaning and young adults depending on the aim of the experiment.

The exact number of mice needed for each experiment will be calculated with a sample size calculation based on historical data from our laboratory and a power assessment. Since the model still needs to be developed an estimation of the amount of mice needed in total is hard and this depends on the number of experiments needed to optimize the model. We estimate that in total 360 mice are needed, 120 of which for the development of the mice model.

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.

Replacement: The goal of this project is to ameliorate the course of chemotherapy-induced mucositis. Mucositis is a multifactorial process which involves many different established contributors (intestinal epithelium, immune system and nutrition) which are hard to mimic in vitro. On top of that we will investigate the role of the intestinal microbiota. Therefore, an accurate in vitro model which combines all these factors is impossible to accomplish. The experiments are clearly too invasive to be able to investigate them in the human situation. Therefore, an animal model of chemotherapy-induced mucositis is needed. The availability of transgenic rats is very limited. To be able to investigate the involvement of specific genes in successful interventions a mice model would be necessary.

Reduction: Experiments during which animals are sacrificed at two or more time points compared to the control group(s) will, when the experimental aim allows this, not be measured on each time point since the control animals do not change during the experiment. Also parameters which do not require the animals to be sacrificed are assessed whenever appropriate, for example citrulline in blood samples and faeces samples.

Refinement: The model will be designed to minimize discomfort and stress for the animals. Anaesthesia and analgesia will be used whenever appropriate to minimize stress and discomfort.

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

The animals are handled often to minimize stress and discomfort during weighing or while being transferred. On top of that, animals are monitored frequently to identify potential discomfort as soon as possible. Anaesthesia and analgesia are given whenever needed. Potential environmental-polluting waste will be collected appropriately.

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.

Several possible interventions are investigated which could potentially ameliorate the course of severe mucositis. The role of microbiota during the development and recovery of chemotherapy-induced mucositis has not yet been extensively investigated. The research that has been done so far showed potential but due to flaws in the experimental design results are not solid enough to allow direct translation to humans. Some studies report nutritional interventions. However, these studies have so far focused on amino acid supplementation, other nutrients or combinations have hardly been studied.

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.

The animals will be housed individually. This is needed since we want to measure food intake, water intake and some experiments require supplementation to the water/food (for example antibiotics).

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.

The mice will be anesthetized with Isoflurane/oxygen by or under supervision of experienced employees. Analgesia will be used if needed.

I. Other aspects compromising the welfare of the animals

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

After administration of chemotherapeutic agent the mice will have several days of lowered intake, weight loss (<10%), diarrhoea and general sickness. We expect the adverse effects of WT mice welfare be similar to the adverse effects of the rat model. Genetic effects present in transgenic mice might possibly compromise welfare of the animals. However, these effects are impossible to describe now since missing gene will be determined based on results of experiments performed in this project. The mice used in this project will be already established transgenic mice strains. How these transgenic strains fare after the administration of chemotherapy could go both ways, depending on the missing gene and the mechanism we want to investigate. A gene maybe need to be inhibited/induced to improve the course of mucositis, a transgenic mice might genetically have an beneficial/worse outcome.

Explain why these effects may emerge.

The goal of our project is to change to course of chemotherapy-induced mucositis, the symptoms of mucositis are an impaired uptake of nutrients and a general sickness. Due to an impaired uptake of nutrients more water will be attracted to the faeces which results in diarrhoea. Combined the general sickness and diarrhoea lead to weight loss.

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

The interventions which are investigated are aimed to prevent occurrence or minimise severity of the mucositis. Also chemotherapy will be given at a dose which ensures the mucositis is self-limiting as it is in patients.

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.

When signs of severe complications, like severe weight loss (>15%) and general severe malaise (behavior, fur, hygiene) the mice will be sacrificed due to humane endpoints. After chemotherapy-injection the mice will be investigated daily.

Indicate the likely incidence.

<5% of the mice which receive chemotherapy

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').

Non-recovery: collection of organs after being sacrificed
Mild: individual housing, transport during experiments, oral gavage, collection of blood from tail vein, injection of intervention (IV,IP,SC), dexa-scan or nuclear magnetic resonance spectroscopy, faecal transfer, repeated collection of blood during glucose absorption test and fasting
Moderate: Administration of chemotherapy/NaCl via injection and experiencing mucositis.
The overall expected level of discomfort is moderate. The animals will be closely monitored after administration of chemotherapy until the end of the experiment.

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.

To be able to investigate the histology of the intestine to evaluate the mucositis and its recovery and to collect the content of the gastrointestinal tract to evaluate the gut microbiota. Other organs are also investigated and harvested depending on the research question

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

Centrale Commissie Dierproeven

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Rijksuniversiteit Groningen
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Centrale Commissie Dierproeven

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www.centralecommissiedierproeven.

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

info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD105002015338

Uw referentie

Bijlagen
1

Datum: 18 JAN. 2016
Betreft: Beslissing Aanvraag projectvergunning dierproeven

Geachte [redacted]

Op 27 november 2015 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "To change the course of chemotherapy-induced mucositis" met aanvraagnummer AVD105002015338. Wij hebben uw aanvraag beoordeeld.

Op 21 december 2015 hebben wij u per mail een aantal vragen gesteld. U heeft op basis van de vragen de aanvraag aangepast en ons op 22 december 2015 de projectaanvraag toegezonden met uw antwoorden daarin verwerkt.

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 "To change the course of chemotherapy-induced mucositis" starten. De vergunning wordt afgegeven van 19 januari 2016 tot en met 1 december 2020. De startdatum wijkt af van uw aanvraag omdat deze in het verleden ligt.
Overige wettelijke bepalingen blijven van kracht.

Procedure
Bij uw aanvraag heeft u een advies van de Dierexperimentencommissie DEC-RUG gevoegd. Dit advies is opgesteld op 24 november 2015. Bij de beoordeling van uw aanvraag is dit advies betrokken overeenkomstig artikel 10a, lid 3 van de wet. Wij hebben de DEC om aanvullende informatie gevraagd. Op 21 december 2015 heeft de DEC gereageerd op onze vragen, het antwoord was voldoende duidelijk.
In het advies van de DEC staat een beperkende voorwaarde vermeld. De noodzaak tot deze voorwaarde is vervallen omdat het projectvoorstel is aangepast en de projectaanvraag nu enkel het gebruik van methotrexaat beschrijft en hiermee aan de voorwaarde van de DEC wordt voldaan. Wij kunnen ons vinden in de inhoud van het advies van de Dierexperimentencommissie. Wij nemen dit advies van de commissie over, inclusief de daaraan ten grondslag liggende motivering. Dit advies en de in de bijlage opgenomen beschrijving van de artikelen van de wet- en regelgeving zijn de grondslag van dit besluit. Met het oog op artikel 10a. van de wet worden aan meerjarige projecten twee algemene voorwaarden gesteld.

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 de rechter kantlijn in deze brief.

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

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

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

Met vriendelijke groet,

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: Rijksuniversiteit Groningen
Adres: A. Deusinglaan 1
Postcode en woonplaats: 9713 AV Groningen
Deelnemersnummer: 10500

deze projectvergunning voor het tijdvak 19 januari 2016 tot en met 1 december 2020 voor het project "To change the course of chemotherapy-induced mucositis"met aanvraagnummer AVD105002015338, volgens advies van Dierexperimentencommissie DEC-RUG
De functie van de verantwoordelijk onderzoeker is [redacted]

De aanvraag omvat de volgende bescheiden:

1. een aanvraagformulier projectvergunning dierproeven, ontvangen op 27 november 2015
2. de bij het aanvraagformulier behorende bijlagen:
   a. Projectvoorstel, zoals ontvangen bij digitale indiening op 22 december 2015;
   b. Niet-technische Samenvatting van het project, zoals ontvangen bij digitale indiening op 22 december 2015;
   c. Advies van Dierexperimentencommissie dd 24 november 2015, ontvangen op 27 november 2015;
   d. De aanvullingen op uw aanvraag, antwoorden via de mail ontvangen op 22 december 2015.

Dierproeven

Naam dierproef	Diersoort	Aantal dieren	Ernst
Chemotherapy-induced mucositis rat model	Ratten (Rattus norvegicus) / wild typw wistar	720	Matig
Chemotherapy-induced mucositis mouse model	Muizen (Mus musculus) / Wild type en genetisch gemodificeerd	360	Matig

Voorwaarden
Op grond van artikel 10a1 lid 2 Wet zijn aan een projectvergunning voorwaarden te stellen
De vergunning wordt verleend onder de voorwaarde dat eventuele go/no go momenten worden genomen met instemming van de IvD.

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.

[redacted]

Van: Info-zbo
Verzonden: donderdag 28 januari 2016 16:39
Aan: [redacted]
Onderwerp: terugkoppeling AVD105002015338

Geachte leden van DEC RUG,

Bij de CCD is een aanvraag tot projectvergunning aangeboden waarover uw Dec advies heeft uitgebracht. Het betreft het project To change the course of chemotherapy-induced mucositis met aanvraagnummer AVD105002015338. Er zijn aan uw DEC. Daarnaast zijn aan de aanvrager een aantal vragen gesteld, daarvan hebben wij gedurende het behandeltraject uw DEC op de hoogte gesteld. Aanvrager heeft de vrager voldoende beantwoord en de documenten aangepast.
De CCD heeft besloten de aanvraag na de aanpassingen te vergunnen en hierbij uw advies gevolgd.

Met vriendelijke groet, [redacted]

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