De ondertekende aanvraagformulieren sturen naar: Erna Dewil, Proefdierencentrum, Herestraat 49 bus 501, 3000 Leuven.

P 164 / 2010

FORMULIER ETHISCHE COMMISSIE
Form Ethical Committee

KATHOLIEKE UNIVERSITEIT LEUVEN

Laboratorium (*laboratory*): Hepatologie

Erkenningsnummer laboratorium (*license number*): LA 1210242

Laboratoriumdirecteur (*laboratory director*):

Naam/*name*	Voornaam/*first name*	Diploma/*degree*
Van Pelt	Jos	Prof. Dr. Ing

Proefleiders (ZAP) (*PI*):

Naam/*Name*	**Voornaam**/*first name*	**Diploma** /*degree*	**Certificaat proefdierkunde**/*certificate lab animal science*
Nevens	Frederik	MD, PhD	☒
.....	.....	.....	☐

Uitvoerende onderzoeker(s) (AAP/BAP) (*researchers and technicians*):

Naam/*Name*	**Voornaam**/*First name*	**Diploma**/*degree*	**Certificaat proefdierkunde**/*certificate lab animal science*
Temmerman	Frederik	MD	☒
Vanderelst	Ingrid		☒
			☐

☒ **Nieuw project** (*new project*)

☐ **Verlenging van of verbonden aan een project (Nr. .....)**/*elongation of project P...*

☐ **Wijziging van een project (Nr.....)**/*change of project P...*

Titel van het onderzoeksproject (*title of the research project*):

Liverpolycystosis: mechanism of human disease. From bench to bedside.

Duur van het project (maximum 4 jaar) (*duration of the project, max 4 years*)
Begindatum (*start date*): 02/08/2010 Einddatum (*end date*): 02/08/2014

Handtekening van de laboratoriumdirecteur/*signature lab director* Datum/*date*

VAN Pelt 6/12/2010

Advies (voorbehouden aan de Ethische Commissie) (*for the Ethical Committee*):

☒ gunstig/*favorable* ☐ gunstig mits aanpassingen/*provided favorable adjustment* ☐ ongunstig/*rejected*

14 XII 2010

Inschatting van pijn, lijden of letsel door de Ethische Commissie/*estimate bij the Ethical Comittee*

☐ geen/*none* ☐ gering/*minor* ☒ matig/*moderate* ☐ ernstig/*severe* ☐ ondefinieerbaar/*undefinable* Datum/*date*:

Commentaar en opmerkingen/*comments and remarks*

Beknopte beschrijving van het onderzoeksproject (vraagstelling, doel van het onderzoek, belang en verantwoording) (*short description of the project, aim, interest and justification*):

The polycystic liver diseases (PCLD) represent a group of genetic disorders, in which cysts occur in the liver only, like in autosomal dominant polycystic liver disease (ADPLD), or occur as well in the liver as in the kidneys as part of autosomal dominant polycystic kidney disease (ADPKD). The natural history of PCLD regardless of etiologic mutation, is strikingly similar. The liver becomes polycystic at a late stage in both disease entities. Most of the patients with PCLD are asymptomatic; however, in a subpopulation of 1-3%, expansion of liver cysts cause invalidating abdominal symptoms. The most common complication in patients with PCLD is extensive hepatomegaly, which may lead to malnutrition and can be lethal. There is no medical treatment approved for PCLD and to date, the only definitive treatment in those patients with large liver volumes is liver transplantation. Therefore, in an era of organ shortage, other therapeutic options to reduce cyst volume need to be assessed, especially because liver function in patients with symptomatic liver polycystosis stays normal. A better understanding of the pathophysiology and the availability of animal models have already facilitated the development of preclinical trials and identification of promising candidate drugs. In this regard, we - and the Mayo Clinic confirmed our data - recently demonstrated that somatostatin analogues binding to the somatostatin receptor subtype 2 (SSTR2) and reducing the cytosolic cyclic adenosine monophosphate (cAMP) concentration ($[cAMP]_{cyt}$) diminish liver volume. We will explore the activity of new promising candidate drugs in rodents with PCLD using a non-invasive method: using MRI. This will strengthen our insights in the pathophysiology and may eventually lead to preclinical trials in patients with PCLD. We will first investigate the effect of pasireotide, a new multi-ligand somatostatin analogue exhibiting high binding affinity to 4 of the 5 somatostatin receptor subtypes (SSTR 1, 2, 3 and 5). Furthermore, we will investigate the role of estrogen receptor blockers since clinical observations suggest an estrogen effect on hepatic cyst growth in patients with PCLD. Finally, we will explore the effect of mTOR inhibitors on liver-cyst volume. The PCK rat, an autosomal recessive *Pkhd1*-gene mutation causes progressive hepatic and renal cyst formation. This rodent will serve as a model of human autosomal recessive polycystic kidney disease (ARPKD) (Charles River Laboratories, France).

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Binnen het project te gebruiken proefdieren (raming van het aantal benodigde dieren voor de bovenvermelde duur van het project) **en inschatting van duur van de proef, duur en graad van pijn, lijden en letsel (zie Referentielijst)** *(species, strain, number of animals to be used, mean duration of the experiment, mean duration of pain, suffering and lasting harm, estimate of pain, suffering and lasting harm)*

Aantal (*number*)	Diersoort en stam (*species and strain*)	Gemiddelde duur van de proef (dagen, weken, maanden) *Mean duration of the experiment*	Gemiddelde duur van pijn, lijden en letsel (dagen, weken, maanden) *Mean duration of pain, suffering and lasting harm*	Graad van pijn, lijden en letsel (geen, gering, matig, ernstig, ondefinieerbaar) *Estimate of pain, suffering and lasting harm (none, low, moderate, severe, undefinable)*
50	Sprague Dawley Strain code 400	weeks	Day peroperative	moderate
50	PCK rat Strain code 453	weeks	Day, peroperative	moderate

Verantwoording van het gespecificeerde *aantal* **benodigde dieren** (*justification for the number of animals)*

The 4 groups will consist of 8 PCK rats because at the end of each group, we want to keep at least 5-6 rats alive. We are aware that rats may die because of manipulation during the study. A minimum of rats is warranted to result in an objective observation at the end.

Geplande ingrepen op de levende proefdieren (*planned experiments on the animals)*

☒ Chirurgische ingrepen/*surgery*
☒ Toediening van stoffen aan niet-verdoofde dieren/ *administration of substances to non-anaesthetised animals*
☐ Klinisch onderzoek van niet-verdoofde dieren/ *clinical investigation of non-anaesthetised animals*
☒ Klinisch onderzoek van verdoofde dieren/ *clinical investigation of anaesthetised animals*
☒ Afname van stoffen of weefsels bij verdoofde dieren/*taking substances or tissue from anaesthetised animals*
☐ Afname van stoffen of weefsels bij niet-verdoofde dieren/ *taking substances or tissue from* non-*anaesthetised animals*
☒ Afname van stoffen of weefsels bij geëuthanaseerde dieren (in vitro studies)/ *in vitro studies*
☐ Conditionering, psychische testen/ *conditioning or psychological studies*
☐ Voederproeven/ *feeding tests*
☐ Andere/*others*: .....

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Toelichting bij de geplande ingrepen op het levende dier – beschrijf chronologisch de geplande manipulaties (*chronological description of the planned interventions/manipulations on the living animal*

SD rats and PCK rat models are available in the Charles River laboratory, France.

* SD rats will be given test doses of the drugs as described above.
  1. Observation, weight, tolerance
  2. Blood sampling after 2 and 4 weeks for analysis of biochemistry

* PCK rats will be divided in 4 groups
  1. Observation, weight, tolerance
  2. blood sampling at week 0 and 4: in the lateral tail
     blood sampling at week 8 (exsanguination)
  3. MRI scanning at week 0, 4 and 8 under anesthesia

Specificeer de nazorg (*specify the after care/postoperative care)*

The rodents will be placed in a warm environment after the manipulations

Gebruikt u tranquillizers ?(*do you use tranquillisers*)

Zo ja, welke? *(if so, which?)*	**Zo neen, waarom niet ?** *(if not, why not)*
	No, because the rodents need to be anesthetized for MRI scanning. Blood sampling at week 0 and 4 will be done by using the lateral tail.

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Gebruikt u analgetica ?(*do you use analgesia and/or postoperative painkilling)*
Gebruikt u postoperatief pijnstilling ?

Zo ja, welke? *If yes, which one(s)*	**Zo neen, waarom niet ?** *If not, why not*
	No, because the rodents need to be anesthetized for MRI scanning. Blood sampling at week 0 and 4 will be done by using the lateral tail.

Gebruikt u anesthetica ? (*do you use anaesthestics)*

Zo ja, welke? ? *If yes, which one(s)*	**Zo neen, waarom niet ? ?** *If not, why not*
Nembutal (pentobarbital)	

Gebruikt u andere postoperatieve producten? *(do you use other postoperative products)*

Zo ja, welke? ? *If yes, which one(s)*	**Zo neen, waarom niet ? ?** *If not, why not*
	No, since the rodents will be anesthetized for MRI scanning. Blood sampling at week 0 and 4 will be done by using the lateral tail.

Bestaan er *in vitro* alternatieven? Waarom worden ze niet gebruikt? *(are there in vitro alternatives, if so, why do you not use them)*

No

Worden de dieren geëuthanaseerd op het einde van het experiment? *(are the animals euthanised after the experiment,)*

Zo ja, specificeer methode van euthanasie? *if so, how*	**Zo neen, waarom niet ?** *if not, why not*
Yes Exsanguination by blood sampling through the aorta	after anaesthesia?

Specificeer de humane eindpunten van uw experiment, m.a.w. wanneer worden de manipulaties stopgezet omdat verder lijden of ongemak voor het dier onnuttig is voor het experiment en dus niet meer te

verantwoorden is? *(specify the humane endpoints, which criteria are used to determine when the animal suffers too much and the manipulations need to be ended)*

These situations will not attend, since the experiments will be conducted as described above.

Inschatting volgens Vlarem van de risicoklasse van het dierexperimentele werk (Enkel in te vullen voor projecten waarbij genetisch gemodificeerde dieren zullen gebruikt of aangemaakt worden of waarbij dieren op een experimentele wijze geïnfecteerd worden met pathogene en/of genetisch gemodificeerde micro-organismen of organismen)/*determine the biosafety risk class of this project (e.g. when viral vectors of infectious material is used)*

Opgelet : u dient zich ervan te vergewissen dat uw activiteiten bekend zijn bij de dienst Bioveiligheid./*make sure that the department of Biosecurity of the university knows about these activities*

☐ Klasse 1/*class 1* ☐ Klasse 2/*class 2* ☐ Klasse 3/*class 3* ☐ Klasse 4/*class 4*