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

P147/2013

KATHOLIEKE UNIVERSITEIT LEUVEN

FORMULIER ETHISCHE COMMISSIE
Form Ethical Committee

=Laboratorium (*laboratory*): Theragnostic Laboratory

Erkenningsnummer laboratorium (*license number*): LA1210224

Laboratoriumdirecteur (*laboratory director*):

Naam/*name*	Voornaam/*first name*	Diploma/*degree*
Ni	Yicheng	MD, PhD

Proefleiders (ZAP) (*PI*):

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

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

Naam/*Name*	**Voornaam**/*First name*	**Diploma**/*degree*	**Certificaat proefdierkunde**/*certificate lab animal science*
Liu	Yewei	MD, PhD Candidate	☒
.....	.....	.....	☐
.....	.....	.....	☐

☒ **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*)**:**

MRI-navigated target selection for diagnosis and treatment of hepatocellular carcinomas

Duur van het project (maximum 4 jaar) (*duration of the project, max 4 years*)
Begindatum (*start date*): 01/10/2013 Einddatum (*end date*): 30/09/2017

Handtekening van de laboratoriumdirecteur/*signature lab director* Datum/*date* 16/09/2013

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

☐ gunstig/*favorable* ☒ gunstig mits aanpassingen/*provided favorable adjustment* ☐ ongunstig/*rejected* 14x2013

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*

Hepato cellular carcinoma induction
daily gavage under paraanesthesia ???
explain

De Voorzitter/ *the Chairman* De Leden/ *the members*

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

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide due to global pandemic of hepatitis B and C viral infections, and this trend is expected to continue for the next 50 years because of the long latency between infection and symptomatic HCC. The prognosis of advanced HCC remains poor, and novel diagnostic and therapeutic strategies are urgently needed. Recently we have developed a novel small molecular dual targeting anticancer theragnostic strategy namely OncoCiDia, which appears to be a generic approach for the management of different kinds of solid tumors as demonstrated by our preliminary results. In order to prove this unique property and to explore the potential applications of OncoCiDia, this PhD project will be conducted.

We will utilize our expertise in noninvasive detection and characterization of chemically (Diethylnitrosamine, DENA) induced rodent HCCs using contrast-enhanced MRI, and exploit our cumulated experiences in experimental oncology to analyze the therapeutic and diagnostic features of OncoCiDia in a full spectrum of HCC lesions. Thus, while exerting its tumoricidal effect, OncoCiDia itself may prove as a imaging biomarker to identify the degree of tumor malignancy and the extent of therapeutic response from each individual HCC lesion.

HCC is particularly common among the population of developing countries. The successful implementation of this project may not only contribute to the global social and economic stability but also strengthen the impact of KU Leuven on the higher educational system there, which in turn will benefit the competence of this university and mutual development cooperation.

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)*
100	SD rats	5 months		Low to moderate
60	SCID mice	Max 1 month		Low to moderate

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

For orthotopical HCC experiment, 100 male SD rats will be included, half of which for methodological validation and the other half for experiments. Specifically, 48 SD rats will be randomized into 4 groups of 12 each after inducing HCCs: Group A receives injections of 2 solvents; Group B receives an injection of Combretastatin A4 phosphate (CA4P) followed by the injection of ^{131}I-Hypericin (^{131}I-Hyp) solvent; Group C receives an injection of CA4P solvent followed by the injection of ^{131}I-Hyp and Group D receives the injections of CA4P and ^{131}I-Hyp.

For xenograft HCC experiment, similar protocol will be adapted but in SCID mice. After the isolation and implantation of rat HCC cells, 12 mice for methodological validation and 48 mice for experiment.

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*

For orthotopical HCC experiment
1. Animal model construction of DENA-initiated rat hepatocarcinogenesis by daily gavage feeding of DENA under gas anesthesia, for about 3 months.
2. In vivo follow-up using MRI studies with contrast media Gd-EOB-DTPA and Mn-DPDP for monitoring HCC growth and tumor characterization, under gas anesthesia.
3. Random groupings based on MRI and drug administration under gas anesthesia, for follow-up of 12 days:
   Group A receives injections of 2 solvents;
   Group B receives an injection of CA4P (10 mg/kg) followed by the injection of ^{131}I-Hyp solvent;
   Group C receives an injection of CA4P solvent followed by the injection of ^{131}I-Hyp (300 MBq/kg);
   Group D receives the injections of CA4P and ^{131}I-Hyp.
4. Scintiscan by gamma camera in rats receiving ^{131}I-Hyp.
5. Serum serum α-fetoprotein (AFP) measurement.
6. Measurement of γ-radiation and ^{131}I-Hyp biodistribution by NaI (TI) gamma counter.

For xenograft HCC experiment
1. Subcutaneous injection of rat HCC cells in SCID mice.
2. In vivo MRI scanning for monitoring tumor growth weekly during 3 weeks.
3. Once the tumor becomes sizable for the treatment, the above protocol (step3~6) for rats will be adapted, but instead of 12 days follow-up, tumor doubling time will be compared as an endpoint (see details below).

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

During and after surgery mice are kept at 37°C under proper anesthesia until natural recovery.

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

Zo ja, welke? *(if so, which?)*	**Zo neen, waarom niet ?** *(if not, why not)*
	Based on our previous experiences in rats and mice, they tolerate and recover well.

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

Zo ja, welke en hoe lang? *If yes, which one(s) and for how long*	**Zo neen, waarom niet ?** *If not, why not*
	For the primary HCC experiment in SD rats, we only analysis the initial stage of HCC (once tumor diameter reaching 5mm), which is asymptomatic and needs no analgesia. For the xenograft HCC experiment in SCID mice, subcutaneous injection will hardly cause pain and needs no analgesia.

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

Zo ja, welke? ? *If yes, which one(s)*	**Zo neen, waarom niet ? ?** *If not, why not*
1. Isoflurin gas anesthesia 2. Nembutal® Sodium Solution (Pentobarbital) if necessary	

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*
	Based on our previous experiences in rats and mice, they tolerate and recover well.

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

There are no in vitro alternatives available in this subject.

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*
By overdose of Nembutal® Sodium Solution (Pentobarbital)	

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)*

In the orthotopic HCC experiment, once tumor diameter reaches 5 mm under the MRI monitoring, rat will be included to receive a 2-day treatment and a 12-day MRI follow-up before euthanasia, which is considered as the humane endpoint.

In the xenograft HCC experiment to compare the tumor doubling time, standardized humane endpoint used to euthanize animals will be decided by MRI monitoring weekly for the first month and twice a week after that until tumor volume doubling.

During the whole experiment, alternate earlier endpoints will be considered whenever one the following situations happens:

1) Rapid loss of weight (>20% of body weight);
2) Tumor exceed 10% of normal body weight;
3) Inability to ambulate (prolonged recumbency);
4) Signs of severe organ or system involvement (Respiratory: rapid or labored breathing; Anaemia; CNS signs: circling, blindness, dementia, and convulsion.)
5) Moribund or pre-moribund state (Define with specific clinical signs and euthanize when reached).

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*

Gebruikt u Tamoxifen?/*Do you use Tamoxifen?* ☐ Ja/*yes* ☒ nee/*no*

Gebruikt u andere chemicaliën (andere dan anesthesie)?/*Do you use other chemicals (others than the anesthesia)?*
☒ Ja/*yes* ☐ nee/*no*

Zo ja, welke: *If yes, which one* Diethylnitrosamine (DENA)

Voor administratieve reden, geef de **naam en het u-nummer van de financiële antenne** die de afhandeling zal doen van de facturen horende bij dit project (*for administrative reasons, please give the name and u-number of the financial antenna that will handle the bills associated with this project)*
: Peter Vermaelen, u0027055

Geef uw **SAP-debiteur** en **krediettype** op voor dit project/ *provide the SAP-debtor and credit type*:
SAP-debiteur (P_- of 405-nummer)/ *SAP-debtor (P_- or 405-number):* P-00014498 KULC
Krediettype (KULC, LRD, VIB, Extern/Spinoff)/*credit type (KULC, LRD, VIB, External/Spinoff)* :
Deze informatie is verkrijgbaar bij uw financiële antenne/ *your financial antenna can give you the requested information.*

Is dit een **Europees project**? *Is this a European project?* ☐ Ja/*yes* ☒ nee/*no*