Begeleidingsformulier aanvraag dierproef DEC- UM [8]
Voorblad werkprotocol CPV [8]
Version Nov. 2005

DECNR[1#]: 2011-092

Ontvangen[#]: 11-08-2011

DEC datum goedkeuring#	Type aanvraag [2]
	Nieuw~~/ Herz.versie / Pilot~~

VROM/GGONR[3]

LNV/CBDNR[4]

Hoofdproject	CARIM	NUTRIM	Hersenen en gedrag	GROW	biomaterialen	Ander UM	Geen UM
Deelproject	1. 2. 3.	1. 2. 3. 4.	1. 2. 3.	1. 2. 3.			.....

Financieel beheerder	.....

Budgetnummer	.....

Titel van het onderzoek:
Reporter gene imaging with SPECT and MRI in tumor metastases

startdatum 01-07-2011 einddatum[9] 01-07-2015 *Duur van de proef[10]:* *16 weken*

	Naam	Tel (+ Tel privé enkel VO, VVO en VM)	E-mailadres	Bevoegd-heid[5]	Cap. groep /afdeling
1.Verantwoordelijk onderzoeker (VO)	.....	.....	.....	Art.9	.....
2. Vervanger VO (VVO)	.....	.....	.....	Art.9	.....
3. VM	.....	.....	.....	Art.9	.....
4. overige uitvoerenden	.....	.....	.....	Art.9	.....
5.	.....	.....		Art.12	.....
6.	.....	.....	.....	Art.9	.....
7.	.....		.....	.....	.....
8.	.....		.....	.....	.....
9.	.....		.....	.....	.....

Diergroep	1	2	3	4	5	6	7
ctrl/exp/sham	exp	exp	exp	exp	exp	exp	exp
Diersoort	01	01	02	01	02	01	02
Stam	Balb/c Nude	Balb/c Nude	RNU	Balb/c Nude	RNU	Balb/c Nude	RNU
Construct / mutatie?	-	-	-	-	-	-	-
Herkomst (leverancier) *	01	01	01	01	01	01	01
Aantal	5	22 + 6	22 + 6	6 + 6	6 + 6	12	12
Geslacht	M/F	M/F	M/F	M/F	M/F	M/F	M/F
Dieren immuuncompetent?	yes	yes	yes	yes	yes	yes	yes
Leeftijd/gewicht	3-7 weken of >20 g	3-7 weken of >20 g	3-7 weken of >200 g	3-7 weken of >20 g	3-7 weken of >200 g	3-7 weken of >20 g	3-7 weken of >200 g
Doel van de proef *	30	30	30	30	30	30	30
Belang van de proef *	01	01	01	01	01	01	01
Toxicologisch onderzoek *	01	01	01	01	01	01	01
Bijzondere technieken *	01	01	01	01	01	01	01
Anesthesie *	04	04	04	04	04	04	04
Pijnbestrijding *	04	04	04	04	04	04	04
Mate ongerief *	03	04	04	05	05	05	05
Toestand dier einde exp*	01	01	01	01	01	01	01

* VHI-codes

Verantwoording

Aanvraag dierproef DEC-UM (**kaders zijn licht flexibel, maar het geheel is max. 5 pag. nov.'05)**

Titel: Reporter gene imaging with SPECT and MRI in tumor metastases

1. Doel van de proef.

The objective of this study is to non-invasively image tumor metastases in-vivo in small animals.

SPECT reporter gene imaging employs certain radiotracers that are specifically trapped in tissues that express certain and characteristic genes and therefore allows visualizing and quantifying gene expression levels in-vivo. The technique is of utmost importance to characterize growth of tumor cells by following gene activity instead of, for example, metabolic activity. Another important application is the characterization of gene activity of tissues after therapeutic interventions such as gene transfection.

In a previous DEC (DEC 2009-047), in-vivo SPECT reporter gene imaging protocols in small animals were established. The SPECT signal was then correlated to gene expression and to tumor size. Also the effect of a pro-drug, ganciclovir, on tumor size, and SPECT signal, was investigated.

In this study, we would like to make the next step towards tumor metastases detection in the small animal body using the capability of our SPECT reporter gene imaging protocols to locate and image them as explained above. The detection of tumor metastasis, originating from the primary induced tumor, is an important tool for small animals to verify the safety of ultrasound induced drug delivery of malignant tumors and can be used for gene delivery or, in future, to support immune response research.

In this project we propose to perform 3 experiments using a tumor cell line that expresses reporter genes:

1. **Blood kinetics.** Determination of blood kinetics of the SPECT radiotracer.
2. **Tumor metastases.** Setting up and characterizing the metastases model.
3. **Tumor inhibition.** The influence of a pro-drug (treatment), ganciclovir, on the tumor metastases will be investigated as a control for setting up this model.
4. **Tumor treatment.** The influence of an anti-cancer drug, ..., on the tumor metastases will be investigated.

The radiotracer is expected to accumulate in the tumor tissue which is imaged and quantified by SPECT. The amount of tracer uptake is the most important readout parameter of all experiments. The MRI scans will add morphological, volumetric and perfusion information. Together with histological information these readouts characterize the tumor model.

.....

2. Maatschappelijke relevantie en/of wetenschappelijk belang

Imaging of gene activity using nuclear imaging is of great importance in evaluating therapeutic interventions and treatment of patients, especially related to new therapeutic options like local gene delivery and regenerative medicine. Besides these direct applications, reporter gene imaging can be a superb research tool in the development of new drugs, drug delivery strategies and treatment options in oncology and cardiology. This technique can also be used as a safety control for cell transplantation experiments if, e.g. the cells are genetically modified with a reporter system. The possibility of tracking small metastases can provide a powerful tool in the design of treatment plans in patients.

3. Alternatieven

Reporter gene imaging with SPECT in small animals was already set up in our lab in a tumor xenograft model. The challenge is now to translate these protocols to image tumor metastases at unknown locations in the body and find exact protocols for future preclinical research on drug delivery and gene delivery, where this technique will be extensively used. The formation of metastases is a complex mechanism that cannot be tested in the in-vitro situation. We therefore do not see any alternatives to avoid animal studies, as for ethical reasons testing in humans cannot be done.

4. Ethische afweging

The development of reporter gene imaging protocols is of great importance for society. It allows the non-invasive imaging of gene expression in–vivo which may lead to new treatment options in oncology, cardiology or metabolic diseases in the human situation. For example the tracking of tumor metastases allows for assessing gene activity at places that are not known beforehand. Also the result of therapeutic interventions such as the up- or down regulation of genes in longitudinal studies over weeks or even months can be investigated. Employing non-invasive reporter gene imaging instead of classical post-mortem pathological methods will reduce the number of animals in future research. Secondly, the obtained information in-vivo can be considered of higher significance than pathological data, as it is more comparable to a later clinical situation.

We are of the opinion that these benefits outweigh the discomfort of the animals in this study.

5. Wetenschappelijke onderbouwing

Reporter gene imaging allows to visualize and quantify gene expression in-vivo. One example is in gene therapy, where a gene of interest with therapeutic relevance is brought into a diseased tissue. The delivery of genes can be accomplished by traditional gene delivery techniques based on viral vectors or using newer techniques such as gene delivery induced by ..... Once taken up into the target tissue (transfection), the question is if the therapeutic gene is actually expressed and shows therapeutic efficacy. Usually, it is impossible to monitor directly the expression level of the therapeutic gene, however, the expression level can be indirectly visualized by fusing a reporter gene to the therapeutic gene behind a common promoter to ensure equal expression levels of both genes. The reporter gene usually encodes for a specific molecule which presence and expression levels can be visualized in-vivo.

The most common example, also used in this study, is the reporter gene expressing the enzyme *Herpes Simplex Virus-1 Thymidine Kinase* (HSV1-TK). This enzyme specifically recognizes and subsequently traps (via phosphorylation) a radiolabeled substrate FIAU (2'-fluoro-2'-deoxy-β-D-5-iodouracil-arabinofuranoside) inside those cells that express the reporter gene. Using radio-labeled FIAU with I-123 allows to localize and quantify the gene expression of HSV1-TK in-vivo with the nuclear imaging technique SPECT. The expression level of HSV1-TK is now representative to the expression of the therapeutic gene coupled to it [1]. In addition, HSV1-TK can also be used as a therapeutic agent as it converts nontoxic prodrugs like ganciclovir or aciclovir to cytotoxic drugs[2]. These nucleotide analogues are phosphorylated by HSV1-TK which inhibits DNA incorporation of dGTP leading to cell apoptosis.

In a previous study (DEC 2009-047), reporter gene imaging protocols for in-vivo SPECT in small animals were established in a tumor xenograft model. A SPECT radiotracer was synthesized and tested. SPECT signal was correlated to gene expression and to tumor size. Also the effect of a pro-drug on tumor size was investigated. Now, we will focus on tumor metastases by imaging gene activity at places not known beforehand. This can provide a powerful tool in the design of treatment plans in patients.

In this study, a human breast adenocarcinoma, triple negative, cell line (MDA-MB-231-LITG) will be used that stably expresses HSV1-TK, firefly luciferase (fluc) and green fluorescent protein (GFP) and as a negative control we will use the wild type MDA-MB-231 cell line for SPECT imaging of gene expression. The tumor cell lines will be injected intra-cardially which will induce the formation of tumors at unknown locations in the body. This process is a good model of tumor metastases and a powerful tool for future research.
Our established ^{123}I-FIAU SPECT imaging protocols from DEC 2009-047 can be used to localize the developed tumors by assessing the expression level of HSV1-TK from the tumor cells. The tumor size and morphology will be also characterized with (contrast enhanced)-MRI. These MRI measurements will give information on the perfusion of the tumor and help identify possible necrotic cores or not perfused areas in the tumors, essential to correlate the SPECT signal and tumor volumes. Also, (contrast-enhanced) ultrasound imaging might be used if time allows within the 4 h anesthesia, mainly in the eventual possibility that the MRI scanner is suddenly not available/working.

In this project we will perform 4 experiments to translate our established SPECT reporter gene protocols to the metastasis situation:

1. First we will determine the blood kinetics of our radiotracer [^{123}I]-FIAU. This experiment was not performed in DEC 2009-047 and adds important information to our studies. After an in-depth revision of current literature we could not find any blood kinetics data for our radiotracer in our mouse model.
2. We will then adapt our protocol to the metastasis situation in experiment 2. Here, a pilot group will be used in which the time it takes for the animals to develop tumors after intracardiac injection will be determined both by SPECT imaging and dissection. After the timepoints are established we will perform the SPECT imaging over a group of animals with statistical significance.
3. Then we will investigate the tumor response on using the pro-drug ganciclovir, which is toxic to HSV-tk expressing cells, by quantifying activity changes from SPECT imaging over time.
4. Finally, the influence of ..... (used in the clinic for the treatment of breast cancer) on the tumor metastases will be investigated by quantifying activity changes from SPECT and tumor changes in size with MRi over time.

6. Wetenschappelijke beoordeling

This DEC proposal is internally assessed and approved by the principal investigator.

Proefdier

7. Proefdier keuze

7a. Soort, stam / herkomst / eindbestemming

Species, strain: mouse: BALB/c Nude mice
rat: RNU rats

The experiment requires immunodeficient rats and mice due to the tumor model.
Supplier: Registered supplier licensed for breeding and supply.
Final destination: Sacrificed at the end of the experimental procedure as outlined in Section 8.

7b. Sexe

It is not expected that the sex will influence the outcome of the study.

7.c. Aantallen

5 + 22 +6 + 6 +6 + 12 = 57 mice
22 + 6 + 6 +6 + 12 = 52 rats
The total numbers of rats and mice is 109.
The experiments will be performed and optimized first with mice. Subsequently, the experiments will be performed in rats. A metastases tumor model and imaging method in both species is necessary to support future research in the area of gene delivery (done in mice) and the studies of treating malignant tumors with ultrasound performed in rats (DEC 2009-126).
Bloodkinetics (experiment 1) will only be performed in mice, since in literature bloodkinetics for the radiotracer in nude rats has already been described. In experiment 2, The metastases tumor model needs to be established and studied in both animal species, because the tumor growth behavior will differ between mice and rats (growth curve, metastasis locations). Also, imaging small tumors in mice and rats will be different because of animal size (loss of radioactivity signal due to tissue absorption) and pharmacokinetics (influencing tumor uptake for instance) (experiment 2 and 3).
If information obtained from the mice groups can be transferred into the rat groups (like number of implanted cells) we will use it and reduce the number of rats accordingly. However we need to plan under the assumption the information might not be directly transferrable between species.

To determine group size for Experiment 1, we chose a power of $\pi = 80\%$ and an error for the test of $\alpha = 0{,}05$. We can then use L. Sachs' formula to determine group size:

L. Sachs formula: $n = 2(z_{\alpha/2} - z_{\pi})^2 * (\sigma/\delta)^2$
It holds: $2(z_{\alpha/2} - z_{\pi})^2 = F$.
For $\alpha = 0{,}05$ and $\pi = 80\%$ it holds that: F=15.7
Thus the Sachs formula can be reduced to: $n = 15.7 * (\sigma/\delta)^2$

In these experiments we are interested in FIAU blood kinetics. We expect a signal change (= δ) in our measurements of around 60% and we expect the distribution to be 32%. Entering this into Sachs' formula gives: $n = 15.7 * (32/60)^2 = 4.5$ animals per group.

To determine group size for Experiments 2 Group 1 and 2, we chose a power of $\pi = 80\%$ and an error for the test of $\alpha = 0{,}05$. We can then use L. Sachs' formula to determine group size:

L. Sachs formula: $n = 2(z_{\alpha/2} - z_{\pi})^2 * (\sigma/\delta)^2$
It holds: $2(z_{\alpha/2} - z_{\pi})^2 = F$.
For $\alpha = 0{,}05$ and $\pi = 80\%$ it holds that: F=15.7
Thus the Sachs formula can be reduced to: $n = 15.7 * (\sigma/\delta)^2$

In these experiments we are interested in FIAU accumulation in tumor cells expressing the HSV1-TK enzyme. We expect a signal change (= δ) in our SPECT scan of around 65% as compared to the negative control (tumor cells not expressing HSV1-TK). In recent experiments (DEC 2009-047) with the same radiotracer and subcutaneous tumors, we found the distribution to be 36%. Entering this into Sachs' formula gives: $n = 15.7 * (36/65)^2 = 4.8$ animals per group.

To determine group size for Experiments 2 Group 3 and Experiments 3 and 4, we chose a power of $\pi = 80\%$ and an error for the test of $\alpha = 0{,}05$. We can then use L. Sachs' formula to determine group size:

L. Sachs formula: $n = 2(z_{\alpha/2} - z_{\pi})^2 * (\sigma/\delta)^2$
It holds: $2(z_{\alpha/2} - z_{\pi})^2 = F$.
For $\alpha = 0{,}05$ and $\pi = 80\%$ it holds that: F=15.7
Thus the Sachs formula can be reduced to: $n = 15.7 * (\sigma/\delta)^2$

In these experiments we are interested in FIAU accumulation in tumor cells expressing the HSV1-TK enzyme. We expect a signal change (= δ) in our SPECT scan of around 80% as compared to the negative control (tumor cells not expressing HSV1-TK). In recent experiments (DEC 2009-047) with the same radiotracer and subcutaneous tumors, we found the distribution to be 55%. Entering this into Sachs' formula gives: $n = 15.7 * (55/80)^2 = 7.4$ animals per group.

Experiment 1. Blood kinetics

First the blood kinetics of the radio-labeled probe [^{123}I]-FIAU will be established, also adding to the SPECT protocol that was established in DEC 2009-047. This experiment was not performed in DEC 2009-047 and adds important information to our studies. After an in-depth revision of current literature we could not find blood kinetics data for our radiotracer in our mouse animal model. For this, the radioactive tracer will be injected into 5 nude mice/rats and blood samples will be taken at 6 time points within 24 hours. Loss of animals during the experiment is estimated to be 5% (based on experiences in the lab, with 1 out of 20 animals we have difficulties to get enough blood for an accurate blood concentration analysis).

Group 1

4.5 mice for blood kinetics, loss = 5%; (a-0.05*a=4.5, a=4.73) →5 mice

Total number of mice for experiment 1: 5

Total number of animals: 5

Experiment 2. Tumor metastases

In this experiment, the metastases tumor model will be established in both nude mice and rats. Tumor cells will be intra-cardially injected while the animal is under anesthesia. Tumor growth will be monitored using FIAU and SPECT and MRI imaging for tumor morphology.

First, scans will be performed in groups of 3 animals at 2 different time points. We will start with groups < 5 because this is a pilot experiment to characterize the tumor development after intracardiac injection (Group 1). If tumor growth cannot be established in the first Group, conditions like number of injected cells and time points will be varied for the next group (Group 2). Once an optimal time point is established, a statistical significant group of nude mice and rats will be injected intra-cardially and scans will be performed with SPECT (^{123}I-FIAU injected as radiotracer) at 3 subsequent time points (Group 3) effectively following tumor development over time in vivo. Loss of animals due to tumor implantation and repeated scans is estimated to be 20% (35% for Group 3).

Group 1

3 mice for tumor model (SPECT), loss = 20% ; (a-0.2*a=3, a=3.75) → 4 mice per time point
3 rats for tumor model (SPECT), loss = 20% ; (a-0.2*a=3, a=3.75) → 4 rats per time point
*Number of mice: 4*2 (group size*time points) = 8*
*Number of rats: 4*2 (group size*time points) = 8*

Group 2

3 mice for tumor model (SPECT), loss = 20% ; (a-0.2*a=3, a=3.75) → 4 mice per time point
3 rats for tumor model (SPECT), loss = 20% ; (a-0.2*a=3, a=3.75) → 4 rats per time point
*Number of mice: 4*2 (group size*time points) = 8*
*Number of rats: 4*2 (group size*time points) = 8*

Group 3

Static SPECT and MRI imaging will be performed with a group of 8 animals. The scans will be performed at 3 time points (before optimal time point, at optimal and after optimal time point) based on the information established in Groups 1 and 2. If the time points have already been successfully measured in animals from Groups 1 or 2 we will their data to reduce the number in Group 3 (keeping the total animal group number to 12).

7.4 mice for tumor model (SPECT), loss = 35% ; (a-0.35*a=7.4, a=11.4) → 12 mice
7.4 rats for tumor model (SPECT), loss = 35% ; (a-0.35*a=7.4, a=11.4) → 12 rats
Number of mice: 12
Number of rats: 12

Total number of mice for experiment 2: 8 + 8 + 12 = 28
Total number of rats for experiment 2: 8 + 8 + 12 = 28

Total number of animals for experiment 2: 28+28=56

Experiment 3: Imaging of Tumor inhibition using a pro-drug and Reporter Gene Imaging with SPECT

In this experiment, the therapeutic effect of the pro-drug ganciclovir on tumor growth will be assessed in the metastasis model. Loss of animals due to the tumor model, anesthesia and drug

injection is estimated to be 35%.

7.4 mice for tumor model (SPECT), loss = 35% ; (a-0.35*a=7.4, a=11.4) → 12 mice
7.4 rats for tumor model (SPECT), loss = 35% ; (a-0.35*a=7.4, a=11.4) → 12 rats
Number of mice: 12
Number of rats: 12

Total number of animals for experiment 3: 12 +12 = 24

Experiment 4: Tumor response imaging to the chemotherapy drug .....
In this experiment, the therapeutic effect of the drug ..... on tumor growth and the changes in the activity signal from SPECT will be assessed in the metastasis model. Loss of animals due to the tumor model, anesthesia and drug injection is estimated to be 35%.

7.4 mice for tumor model (SPECT), loss = 35% ; (a-0.35*a=7.4, a=11.4) → 12 mice
7.4 rats for tumor model (SPECT), loss = 35% ; (a-0.35*a=7.4, a=11.4) → 12 rats
Number of mice: 12
Number of rats: 12

Total number of animals for experiment 4: 12 +12 = 24

Dierproef

8. Experiment

Reporter gene imaging of tumors at unknown locations with SPECT will be tested using the cell line MDA-MB-231-LITG, stably expressing the enzyme HSV1-TK, as well as fluc and GFP. As a control, the wild type MDA-MB-231-WT cell line will be used, which does not express any of these reporter proteins. We also would like to perform (contrast-enhanced) ultrasound and optical imaging if time allows during anesthesia and never for longer than 4h. Ultrasound is especially important if by any reason the MRI scan is not is not possible because we can always retrieve volume and perfusion information.

Since this pilot study is needed for future studies in which mice and rats will be used, experiments will be performed both in nude mice and rats. The experiments will be performed first with mice. Before performing an experiment, it will always be critically assessed whether results from both animal species are needed. We will base our initial time points for tumor growth after intracardial injection from published work.

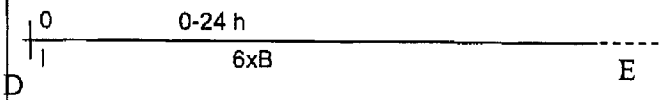
Experiment 1: Blood kinetics

The blood kinetics of the radiolabeled probe [$^{123/125}$I]-FIAU will be established.

Animals will be injected with [$^{123/125}$I]-FIAU into the tail vain. Within 24 hours, blood will be withdrawn from the vena saphena at 6 different time points (maximum 20 µl per time point) in order to determine radioactivity in the blood on a γ-counter. This experiment was not performed in DEC 2009-047 and adds important information to our studies for publication. After an in-depth revision of current literature we could not find any blood kinetics data for our radiotracer in our animal models.

At the end of the experiment the animals will be euthanized under anesthesia (t=24h) and organs will be taken out for bio-distribution and histological studies.

Time line:



D: addition of 0.1% NaI or KI solution to drinking water 24-72 hours prior to injection of radiotracer

I: injection of tracer ([^{123}I]-FIAU)

B: blood withdrawal

E: Euthanization

Experiment 2: Tumor metastases

In this experiment the metastasis tumor model will be established in nude mice and SPECT protocols for this situation will be optimized. Animals will receive intra-cardiac injection with 10^5-10^7 MDA-MB-231-LITG tumor cells (in sterile PBS), under anesthesia. We will base our initial time points for tumor growth after intra-cardial injection from published work [4]. Tumor development will be followed by SPECT and (contrast-enhanced) MRI (or ultrasound). 24h-72h before tracer injection a solution of NaI or KI can be added to the drinking water to block the thyroid uptake of radioactive iodide (exact time point will come from results of DEC 2009-047).

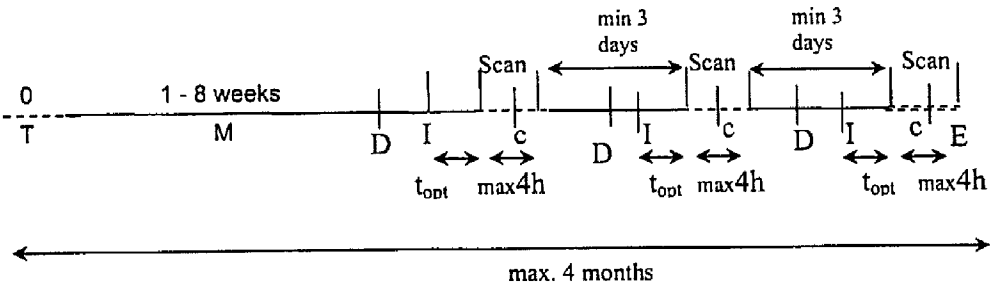
Tumor development will be followed by a second and third SPECT and (contrast-enhanced) MRI (or ultrasound) scan with a minimum of 3 days in between scans. The animals will be euthanized after the last scan, and the blood, urine and organs will be used for bio-distribution and histological studies.

Group 1:
The tumor model for metastasis will be established in group 1 and 2. After allowing some time for tumor growth at unknown locations, [^{123}I]-FIAU will be injected intravenously in the tail vein and subsequently static full body SPECT and (contrast-enhanced) MRI (or ultrasound) scans will be performed. We will base our initial time points for tumor growth after intracardial injection from published work [4].
In DEC 2009-047, the tumor model for subcutaneous injection was established for MDA-MB231-LITG cells. These results serve as a guideline for the optimal time point for the SPECT scans. Animals will be divided into 2 groups and will be scanned parallel to each other with some days in between (e.g. 3 mice start the first scan on day X, and 3 mice start the first scan on day X+7, and the whole procedure will be equal for both groups). This is done to include more time points thus better optimizing the metastasis tumor model. Maximum time under anesthesia is 4 hours. The animals will recover from the first SPECT and (contrast-enhanced) MRI (or ultrasound) scan and the whole scanning procedure (including [^{123}I]-FIAU injection and clinically approved MRI contrast agents or ultrasound contrast agents) will be repeated to investigate tumor development. A third scan will also be performed. After the third scan, animals will be euthanized and organs will be taken out for bio-distribution and histological studies

Group 2
In case no satisfying tumor model can be established in group 1, group 2 can be used for the same experiment but parameters like the number of injected cells or time before first SPECT scan post-injection of tumor cells can be varied. In case group 1 was successful, group 2 will not be used.

Group 3
Static full body SPECT and (contrast-enhanced) MRI (or ultrasound) scans will be performed. Scans will take place just before, at and after the optimal time point after intra cardiac tumor injection established in group 1 and 2. Maximum time under anesthesia is 4 hours. The animals will recover from the first SPECT and (contrast-enhanced) MRI (or ultrasound) scan and the whole scanning procedure (including [^{123}I]-FIAU injection and clinically approved MRI contrast agents or ultrasound contrast agents) will be repeated later to investigate tumor development. A third scan will also be performed. After the third scan, animals will be euthanized and organs will be taken out for bio-distribution and histological studies

Time line for all groups:

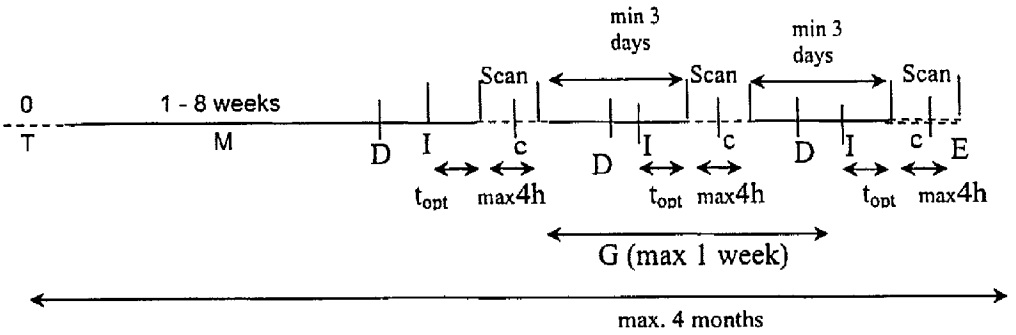


T: Injection tumor cells *MDA-MB231-LITG*
M: tumor growth
t_{opt}: optimal time for tracer uptake post injection
D: addition of 0.1% NaI or KI solution to drinking water 24-72 hours prior to injection of radiotracer
I: injection of tracer ([^{123}I]-FIAU)
C: injection contrast agent for MRI
Scan: SPECT, MRI (within 4 hours of anesthesia)
E: Euthanization
---: under anesthesia.

Experiment 3: Imaging of tumor inhibition using a pro-drug and reporter gene imaging with SPECT

This experiment aims to determine the effect of the pro-drug ganciclovir on tumor development. This is an important control to verify the developed model and protocols.
Animals will be intra-cardially injected with 10^5-10^7 MDA-MB-231-LITG tumor cells (in sterile PBS), under anesthesia. At determined time point after intra-cardiac tumor cell injection (established in experiment 2) the HSV1-TK expression in tumors will be imaged with [^{123}I]-FIAU using SPECT followed directly by a (contrast enhanced) MRI (or ultrasound scan) to determine the tumor morphology and volume. 24h-72h before tracer injection a solution of NaI or KI can be added to the drinking water to block the thyroid uptake of radioactive iodide (exact time point will come from results of DEC 2009-047). The optimal dose and activity of [^{123}I]-FIAU and time of scan after injection of the tracer has been previously determined in experiments from DEC 2009-047. Animals will recover from the anesthesia and ganciclovir treatment will start (i.p. injection up to 2x/day for a maximum of 1 week (Max. dose of 50 mg/kg) according to the published protocol. Animals will be scanned up to three times with SPECT and (contrast-enhanced) MRI (or ultrasound). After the final scans, the animals will be euthanized for bio-distribution and histological examination. In between the scans there will be a minimum of 3 days for recovery.

Time line of experiment 3:



T: Injection tumor cells *MDA-MB-231-LITG*
M: Measurement tumor growth
G: ganciclovir injection (i.p.) up to 2x daily for a maximum of 1 week
D: addition of 0.1% NaI or KI solution to drinking water 24-72 hours prior to injection of radiotracer
t_{opt}: optimal time for tracer uptake post injection
I: injection of tracer ([^{123}I]-FIAU)
C: injection contrast agent for MRI or ultrasound
Scan: SPECT, MRI, optical imaging or ultrasound imaging (within 4 hours of anesthesia)
E: Euthanization
---: under anesthesia.
At least 3 days in between scans

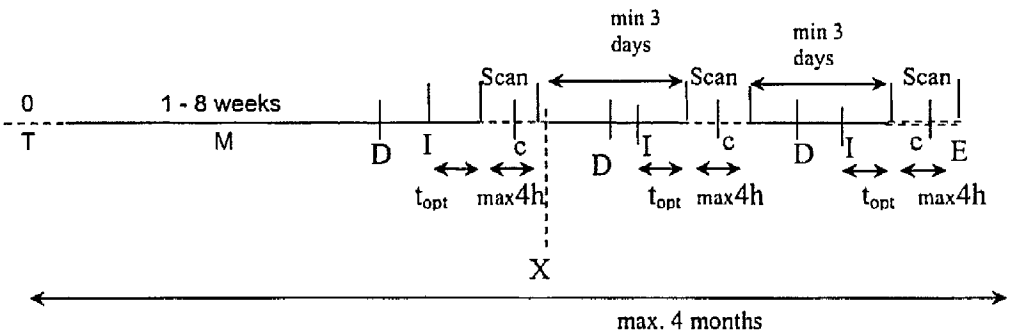
<u>Experiment 4: Tumor response imaging to the chemotherapy drug .....</u>

This experiment aims to determine the effect of..... on tumor development. This is an important experiment to verify the developed model and protocols as ..... is a commonly used drug for the treatment of triple negative breast cancers in the clinic.

Animals will be intra-cardially injected with 10^5-10^7 MDA-MB-231-LITG tumor cells (in sterile PBS), under anesthesia. At determined time point after intra-cardiac tumor cell injection (established in experiment 2) the HSV1-TK expression in tumors will be imaged with [^{123}I]-FIAU using SPECT followed directly by a (contrast enhanced) MRI (or ultrasound scan) to determine the tumor morphology and volume. 24h-72h before tracer injection a solution of NaI or KI can be added to the drinking water to block the thyroid uptake of radioactive iodide (exact time point will come from results of DEC 2009-047). The optimal dose and activity of [^{123}I]-FIAU and time of scan after injection of the tracer has been previously determined in experiments from DEC 2009-047. After the first imaging scan animals will be injected with ..... (when possible we will use .....) to start the treatment: 1x i.v. injection (Max. dose of 5 mg/kg). Animals will be scanned up to three times with SPECT and (contrast-enhanced) MRI (or ultrasound). After the final scans, the

animals will be euthanized for bio-distribution and histological examination. In between the scans there will be a minimum of 3 days for recovery.

Time line of experiment 4:



T: Injection tumor cells *MDA-MB-231-LITG*
M: Measurement tumor growth
X: i.v. ..... (either awake or under anesthesia via canula)
D: addition of 0.1% NaI or KI solution to drinking water 24-72 hours prior to injection of radiotracer
t_{opt}: optimal time for tracer uptake post injection
I: injection of tracer ([^{123}I]-FIAU)
C: injection contrast agent for MRI or ultrasound
Scan: SPECT, MRI, optical imaging or ultrasound imaging (within 4 hours of anesthesia)
E: Euthanization
---: under anesthesia.
At least 3 days in between scans

9. Experimentele condities

9a. Anesthesie

The animals will be placed under anesthesia by administration of isoflurane (induction 2-3%, maintenance 1-2%) in filtered compressed air (complying with the requirements for Medical Air) 0.4 l/min as carrier gas in an anesthesia container, and subsequently maintained under anesthesia in the scanner using a face mask.

9b. Pijnbestrijding

Under normal circumstances, these procedures will not cause any pain to the animals (intra-cardiac injection of tumor cells might be performed together with a painkiller, if necessary) and therefore pain medication is not indicated as standard. However, in the unlikely event that the animals do exhibit any signs of pain, medication (Temgesic = buprenorfine or Rimadyl = carprofen) will be administered in consultation with the article 12 officer. For the euthanization via cervical dislocation pain medication will be given before the procedure.

9c. Euthanasie en Humane eindpunten

- The animals will be euthanized under anesthesia by means of cervical dislocation (or heart puncture) by the hands of an experienced article 12 officer. Alternatively, an automatic O_2/CO_2 chamber euthanasia device will be used or an overdose of isoflurane or pentobarbital.
- The code of practice in cancer research (Inspectie W&V, 1999) [3] will be used as guideline for responsible endpoints. When a weight loss of more than 15% is observed, tumors reach a size of 1500 mm3 (mice) or 35 cm3 (rats), the behaviour and locomotion of the animal becomes seriously abnormal, ulcerations, infections or other serious clinical symptoms are present, the animal will be euthanized prematurely after consultation with the article 12 or 14 officer.

Zorg

10a. Ongerief

Experiment 1

Procedure	Repetition	Duration	Discomfort code
Intravenous injection of radiotracer awake	1	2 min	03
Blood sampling via vena saphena	6	1 min	03
Euthanasia under anesthesia	1	5 min	02
		Total	03

Estimation of total suffering: moderate (code 03)

Experiment 2

Procedure	Repetition	Duration	Discomfort code
Intra-cardiac injection of tumor cells, under anesthesia, resulting in tumor growth	1	max 1 h	04
Cannulation of the tail vein under anesthesia and injection of radiotracer/contrast agent through the cannula	3	10 min	04
Living with a tumor	1	Max. 16weeks	03
SPECT and/or MRI and/or ultrasound imaging under anesthesia, followed by recovery	3	max. 4 hours	03
Euthanasia under anesthesia	1	5 min	02
		Total	04

Estimation of total suffering: moderate/severe (code 04)

Experiment 3

Procedure	Repetition	Duration	Discomfort code
Intra-cardiac injection of tumor cells, under anesthesia, resulting in tumor growth	1	max 1 h	04
Cannulation of the tail vein under anesthesia and injection of radiotracer/contrast agent through the cannula	3	10 min	04
Living with a tumor	1	Max. 16 weeks	03
SPECT and/or MRI and/or ultrasound imaging under anesthesia, followed by recovery	3	max. 4 hours	03
Intraperitoneal injection ganciclovir	14	1 min	05
Euthanasia under anesthesia	1	5 min	02
		Total	05

Estimation of total suffering: severe (code 05)

Experiment 4

Procedure	Repetition	Duration	Discomfort code
Intra-cardiac injection of tumor cells, under anesthesia, resulting in tumor growth	1	max 1 h	04
Cannulation of the tail vein under anesthesia and injection of radiotracer/contrast agent through the cannula	3	10 min	04
Living with a tumor	1	Max. 16 weeks	03
SPECT and/or MRI and/or ultrasound imaging under anesthesia, followed by recovery	3	max. 4 hours	03
Intravenous injection of ..... and therapy effect	1	1 min	04
Euthanasia under anesthesia	1	5 min	02
		Total	05

Estimation of total suffering: moderate/severe (code 04)

10b. Welzijnsevaluatie

An experienced team performed already several studies with tumor models and imaging (SPECT and MRI experiments) and evaluated the level of suffering of mice and rats. The level of suffering is expected not to exceed 05. The principal investigator or article 12 officers evaluate after each experiment the condition and level of suffering recovering from anesthesia. The animals are checked daily by an article 12 officer and their condition is documented.

11. Verzorging en huisvesting

..... They will be cared for according to standard practices by article 12 employees of the University of Maastricht. In particular, it will be taken into account that these are immunodeficient animals and that GGO regulations should be applied. In case of any unforeseen events which may affect the animal welfare the article 12 officers will be notified immediately.

12. Deskundigheid

The article 12 officers will handle the animals during the experiment. They have much experience in the care, anesthesia, injection and euthanasia of mice and rats. They will monitor the animals during the SPECT and MRI scans. .....

13. Standard Operation Procedures (SOP)

.....

Relevante literatuur

.....

University Maastricht

Faculty of Health, Medicine
and Life Sciences

Dierexperimenten Commissie

DEC

voorzitter
p/a Secretariaat DEC-UM
Postbus 616
NL-6200 MD Maastricht
Telefoon:

Aan:

Uw referentie: Onze referentie : Maastricht, 29-06-2011

Geachte Onderzoeker,

Uw projectaanvraag: "*Reporter gene imaging with SPECT/CT and MRI in tumor metastases*", is op de DEC vergadering van 24 juni 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

- De DEC verzoekt bij punt 6 "**The** research proposal"aan te passen in "**This DEC** proposal".
- Bij punt 7c vraagt de DEC zich af waarom alle experimenten, bij zowel muizen als ratten, dienen te geschieden.
- Waarom is een power van 90 noodzakelijk?.
- Bij punt 7c-experiment 1- staat dat er met een uitval van 5% wordt gerekend. In de tekst bij Group 1, heeft men het over loss=10%.
  De DEC verzoekt de tekst in overeenstemming te brengen en niet tussentijds af te ronden (dit zou 1 dier per groep minder betekenen)
- De DEC merkt op dat de delta en simga, gebruikt ter berekening van de groepsgroottes in experiment 1, geen betrekking hebben op de parameters van de bloedkinetiek metingen. Gaarne aanpassen.
- Bij punt 10a verzoekt de DEC het leven met een tumor ook bij het ongerief te vermelden.

Gelieve eventuele vragen te beantwoorden in **een brief en indien noodzakelijk Uw project aan te passen en duidelijk de aanpassingen grijs te markeren.**
Uw project staat bij de DEC geregistreerd onder nummer **2011-092**, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

From:
Sent: donderdag 7 juli 2011 17:12
To:
Subject: RE: Project 2011-092-w, respons
Attachments: 20110707_2011-092_Reporter gene imaging_intracardiac_MQ-348-11.docx

Beste secretaris DEC /

In antwoord op de vragen/opmerkingen van de DEC naar aanleiding van projectaanvraag 2011-092:

De DEC heeft een aantal vragen en opmerkingen:

- De DEC verzoekt bij punt 6 "**The** research proposal"aan te passen in "**This DEC** proposal".
  - It has been corrected in the DEC text.

- Bij punt 7c vraagt de DEC zich af waarom alle experimenten, bij zowel muizen als ratten, dienen te geschieden.
  - Our laboratory works with both mice and rats and we need to establish the imaging protocols and tumor metastases model for both mice and rats for future studies. If information obtained from the mice groups can be transferred into the rat groups (like number of implanted cells or tumor growth times) we will use it and reduce the number of rats accordingly. However we need to plan under the assumption the information might not be directly transferable between species. After reviewing the literature we do not know of a study showing the blood kinetics of the FIAU probe in the Balb/c nu/nu, however we did find a publication with the blood kinetics for RNU rats). We would therefore like to remove the rats from the blood kinetics group in DEC2011-092.
    Text has been added to explain the necessity of using both mice and rats.

- Waarom is een power van 90 noodzakelijk?.
  - In experiment 1, bloodkinetics, indeed a power of 80% is sufficient. The text has been corrected accordingly.
  - In experiment 2 and 3, We will image the growth of tumor metastases in unkown locations and unknown sizes and the smaller the metastase the more confident we need to be with the image. The results from this study will be used in future studies with therapy applications. The strength of this methodology is the non-invasive long term imaging and quantification re-enforcing the need to have high power values in our results.

- Bij punt 7c-experiment 1- staat dat er met een uitval van 5% wordt gerekend. In de tekst bij Group 1, heeft men het over loss=10%.
  De DEC verzoekt de tekst in overeenstemming te brengen en niet tussentijds af te ronden (dit zou 1 dier per groep minder betekenen)
  - There was a typing error in the text. The calculation was made using the 5% value but the text before had 10% written. It has been corrected on the original text.
  - We could not find were we roundoff the number, causing a difference of 1 animal.

- De DEC merkt op dat de delta en simga, gebruikt ter berekening van de groepsgroottes in experiment 1, geen betrekking hebben op de parameters van de bloedkinetiek metingen. Gaarne aanpassen.
  - This has been corrected on the original DEC text.

- Bij punt 10a verzoekt de DEC het leven met een tumor ook bij het ongerief te vermelden.
  - It has been corrected in the DEC text.

Bovenstaande antwoorden zijn in de aanvraag aangepast en in grijs gemarkeerd.
Hopende hiermee afdoende de vragen van de DEC te hebben beantwoord,

12-7-2011

Met vriendelijke groet, Iris

Phone
Email: i
www.

I
Sent: Thursday 30 June 2011 8:52
To:
Subject: Project 2011-092-w

Geachte onderzoeker,
Uw projectaanvraag is in de DEC-UM – vergadering van **24 juni 2011** besproken.
De uitslag treft u aan in bijgaand attachment.
Voortaan zult u uit efficiency overweging geen schriftelijke bevestiging meer ontvangen per post wanneer het een wijzigingsbrief betreft.
De DEC verzoekt U in een brief de vragen van de DEC te beantwoorden en de wijzigingen in het protocol duidelijk grijs te markeren, zodat het bij het kopiëren ook zichtbaar is.
Wannneer uw project is aangehouden (dit staat altijd in de brief) moet u er rekening mee houden dat de herziene terug moet naar de gehele commissie. Uw herziene versie dient uiterlijk 5 juli 2011 binnen te zijn voor de vergadering van 15 juli op het secretariaat.dec.
De eerstvolgende vergadering na 15 juli, is 26 augustus 2011.
Met vriendelijke groet namens DEC-UM:

Ambtelijk Secretaris Dierexperimentencommissie

Postbus 616 6200 MD Maastricht

E-mail:

Werktijden: Ma-Di-Wo-Don van 08.00 uur tot 16.00 uur

From:
Sent: woensdag 20 juli 2011 16:28
To:
Cc:
Subject: RE: Project 2011-092-w, respons
Attachments: 20110720_2011-092_Reporter gene imaging_intracardiac_MQ-348-11_aangepast.docx

Beste

- Na onderling overleg hebben we besloten de power aan te passen naar 80%, omdat we geen aanvullende zwaarwegende argumenten voor 90% konden bedenken. Naar aanleiding van zeer recente resultaten (DEC 2009-047) met dezelfde radiotracer in een tumormodel, hebben we de geschatte spreiding van 30% aangepast naar de gevonde spreiding van 36%. Hierdoor komt de groepsgrootte op n=4,8.

- De aantallen dieren zijn herberekend zonder tussendoor af te ronden.

Het aangepaste protocol 2011-092 is bijgevoegd.

Met vriendelijke groet, I

Phone +

From:
Sent: Wednesday 13 July 2011 15:17
To:
Subject: FW: Project 2011-092-w, respons

Geachte onderzoeker, beste

De DEC heeft je herziene versie behandeld, maar heeft nog de volgende vraag/opmerking:

-De DEC wenst een goede onderbouwing voor een power van 90%.

-Punt 7c- Instead of calculating drop out over the calculated group size of 4.5, this is performed on the prematurely rounded of number of the groupsize (5). Final groupsze should therefore be calculated as follows: n=4.5, drop-out 20%: 4.5/0.8= 5.6, so n=6 (and not 7) per group. Please adapt this and similar cases accordinly.

Graag je reactie.

Met vriendelijke groet namens DEC-UM:

Maastricht University

Faculty of Health, Medicine and Life Sciences

Aan:

Ons kenmerk	*Doorkiesnummer*	*Maastricht* 27-07-2011

Project: *Reporter gene imaging with SPECT/CT and MRI in tumor metastases.*

Verantwoordelijk onderzoeker (VO):

Namens de Vergunninghouder van de DEC-UM, delen wij u mede dat voornoemd project aan de ethische toetsingscriteria voor proefdiergebruik voldoet.
De DEC maakt geen bezwaar tegen uitvoering van dit project zoals aangevraagd en geeft een **positief advies**.

Projectnummer:	**2011-092**
Diersoort:	**muis en rat**
Aantal dieren:	**33 muizen en 28 ratten**
Einddatum:	**27-07-2015**

Uw project staat bij de DEC en CPV geregistreerd onder bovenstaand nummer. Gelieve dieren, die voor dit project bestemd zijn, ook onder dit nummer aan te vragen.

Voorzitter DEC-UM

Vicevoorzitter DEC-UM

DEC-UM
Voorzitter DEC-UM

p/a secretariaat DEC-UM

Secretariaat DEC-UM

Bezoekadres

Postadres
Postbus 616
6200 MD Maastricht