Begeleidingsformulier aanvraag dierproef DEC- UM [8]
Voorblad werkprotocol CPV [8]

Version Nov. 2005

DECNR[1#]: 2011-094

Ontvangen[#]: 12-07-2011

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

VROM/GGONR[3]	LNV/CBDNR[4]

Hoofdproject	CARIM	NUTRIM	Hersenen en gedrag	GROW		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:

Assessment of pain relief of prostate cancer bone metastasis treated with .....

startdatum 1-7-2011 einddatum[9] 1-7-2015 *Duur van de proef[10]: 5 months*

	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) GGO[7]					
4. overige uitvoerenden	.....	.....		Art.12	.....
5.	.....	.....	.....	Art.9	.....
6.	.....	.....	.....	Art.9	.....
7.	.....	.....	.....	.....	.....
8.	.....	.....	.....	.....	.....
9.	.....	.....	.....	.....	.....

Diergroep	1	2	3	4	5	6	7
ctrl/exp/sham	Exp	Exp	Exp	Exp	Exp	Sham	Ctrl
Diersoort	02	02	02	02	02	02	02
Stam	Copenhagen rats	Copenhagen rats	Copenhagen rats	Copenhagen rats	Copenhagen rats	Copenhagen rats	Copenhagen rats
Construct / mutatie?	-	-	-	-	-	-	-
Herkomst (leverancier) *	01	01	01	01	01	01	01
Aantal	30	60	60	5	11	10	10
Geslacht	M	M	M	M	M	M	M
Dieren immuuncompetent?	yes	yes	yes	yes	yes	yes	yes
Leeftijd/gewicht	>200 g	>200 g	>200 g	>200 g	>200 g	>200 g	>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 *	05	05	05	05	05	05	04
Toestand dier einde exp*	01	01	01	01	01	01	01

* VHI-codes

Diergroep	8	9	10	11	12	13	14
ctrl/exp/sham	Exp	Exp	Exp	Exp	Exp	Exp	Exp
Diersoort	02	02	02	02	02	02	02
Stam	Copenhagen rats	Copenhagen rats	Copenhagen rats	Copenhagen rats	Copenhagen rats	Copenhagen rats	Copenhagen rats
Construct / mutatie?	-	-	-	-	-	-	-
Herkomst (leverancier) *	01	01	01	01	01	01	01
Aantal	5	11	11	11	11	11	11
Geslacht	M	M	M	M	M	M	M
Dieren immuuncompetent?	yes	yes	yes	yes	yes	yes	yes
Leeftijd/gewicht	>200 g	>200 g	>200 g	>200 g	>200 g	>200 g	>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 *	05	05	05	05	05	05	05
Toestand dier einde exp*	01	01	01	01	01	01	01

Diergroep	15	16	17	18	19	20
ctrl/exp/sham	Exp	Exp	Exp	Exp	Exp	Exp
Diersoort	02	02	02	02	02	02
Stam	Copenhagen rats	Copenhagen rats	Copenhagen rats	Copenhagen rats	Copenhagen rats	Copenhagen rats
Construct / mutatie?	-	-	-	-	-	-
Herkomst (leverancier) *	01	01	01	01	01	01
Aantal	11	11	10	10	30	30
Geslacht	M	M	M	M	M	M
Dieren immuuncompetent?	yes	yes	yes	yes	yes	yes
Leeftijd/gewicht	>200 g	>200 g	>200 g	>200 g	>200 g	>200 g
Doel van de proef *	30	30	30	30	30	30
Belang van de proef *	01	01	01	01	01	01
Toxicologisch onderzoek *	01	01	01	01	01	01
Bijzondere technieken *	01	01	01	01	01	01
Anesthesie *	04	04	04	04	04	04
Pijnbestrijding *	04	04	04	04	04	04
Mate ongerief *	05	05	05	05	04	04
Toestand dier einde exp*	01	01	01	01	01	01

Aanvraag dierproef DEC-UM

Titel: Assessment of pain relief of prostate cancer bone metastasis treated with .....

1. Doel van de proef.

The aim of this study is to evaluate the therapeutic efficacy and effect of ..... as palliative treatment for bone metastases from prostate cancer. For this aim, we will need to develop a prostate cancer bone metastases model, develop and optimize protocols for imaging of bone metastases, and furthermore develop ..... for treating bone metastases.

..... is a relatively new and non-invasive technique currently being tested in clinical trials to treat benign tumors. In recent studies, the application of ..... was extended to palliative treatment of bone metastases by ..... Positive outcome of the published palliative treatment results have sparked many interest to apply ..... for this purpose. However, little is known about the efficiency and effects of .....-mediated palliative treatments to patients. Therefore, the purpose of the proposal is to set-up all necessary animal models and procedures for ..... treatment and finally, evaluate the efficiency and effects of ..... treatment.

The experiments are divided into four parts:

1) Bone metastases model will be set-up by intra-osseous injection of prostate cancer cells.
2) Development and optimization of ..... parameters for imaging and treatment of bone metastases.
3) Therapeutic evaluation of ..... palliative treatments.
4) Therapeutic evaluation of ..... in combination ..... to further improve palliative pain management.

The main readout will be the outcome of behavior tests which will give a qualitatively and quantitatively assessment of the degree of pain relief. With this readout the efficiency and the effect of the palliative treatment will be evaluated and complemented by, using MRI to assess the size and morphology of the tumors, single-photon emission computed tomography (SPECT) to assess the bone lesions activity at the metastases, micro-computed tomography (uCT) to evaluate the micro-architectural changes in bones and to provide anatomical information for SPECT, blood test to assess the immune response and prostate specific antigen (PSA) level and histology for bio-analysis or tumors and organs.

2. Maatschappelijke relevantie en/of wetenschappelijk belang

As reported in the Global Cancer Statistics published in 2011, prostate cancer is the second most frequently diagnosed cancer and the sixth leading cause of cancer death in males, accounting for 14% (903,500) of the total new cancer cases and 6% (258,400) of the total cancer deaths in males in 2008. Of all the advanced prostate cancer patients, 80% are diagnosed with bone metastases. Patients with bone metastases have greatly reduced quality of life. They suffered from severe pain as a result of pathological fractures, hypercalcemia and spinal cord compression. External-beam radiation therapy (EBRT) and chemotherapy are treatment options available. However, 20-30% patients treated with EBRT do not experience pain relief and recurrence pain appears in 27% of the cases. Moreover, chemotherapy often comes with severe side effects. Hence, improved treatment options are necessary. ..... This treatment option will greatly improve patients' quality of lives.

3. Alternatieven

.....procedures will first be tested on phantoms to make an estimation of optimum parameters. However, *in vivo*, these parameters will change due to ..... Hence, animals are needed for the

protocol optimization. ....., animal experiments are needed as due to medical and ethical reasons, testing in humans cannot be done.

4. Ethische afweging

In order to improve the quality of life of cancer patients, it is of upmost importance to improve the treatment efficacy and reduce the treatment burden on patients. ..... Therefore, the use of animals is necessary to develop and optimize the treatment protocols and eventually, to obtain information regarding the long term effects of the treatments. By taking into consideration the numerous numbers of patients who will benefit from the treatment, we believe that the benefits outweigh the discomfort of the animals used in this study.

Wetenschap

5. Wetenschappelijke onderbouwing

Prostate cancer is the second most frequently diagnosed cancer and the sixth leading cause of cancer death in males, accounting for 14% (903,500) of the total new cancer cases and 6% (258,400) of the total cancer deaths in males in 2008 [1]. 80% of the advanced prostate cancer patients are diagnosed with bone metastases and have osteoblastic bone lesions. Patients with bone metastases have greatly reduced quality of life. They suffered from severe pain as a result of pathological fractures, hypercalcemia and spinal cord compression. As a result, palliative treatment with management of pain and improve quality of life remains the main goal of therapy. Currently, treatment options include systemic drug therapy, surgery, cryotherapy, radiofrequency ablation, radiation therapy and others. External-beam radiation therapy (EBRT) remains the current standard of care for patients. However, 20-30% patients treated with EBRT do not experience pain relief and recurrence pain appears in 27% of the cases. ..... are more effective, but these procedures are normally invasive. Moreover, efficacy of systemic drug therapy is often hampered by the side effects of the drugs, thereby resulting in administration of suboptimum dosage. Hence, improved treatment options are necessary.

.....

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: Copenhagen rats
Supplier: Registered supplier licensed for breeding and supply.
Final destination: Sacrificed at the end of the experimental procedure.

7b. Sexe
Male rats are needed as the study is to investigate the efficacy of palliative treatment of bone metastases from prostate cancer.

7.c. Aantallen
30+ 180 + 105+44 = 359 rats

Experiment 1. Pilot study to establishing tumor model of bone metastases from prostate cancer

In this experiment, the tumor model will be established in rats by intra-osseous injection of MAT-LyLu tumor cells. This cell line is chosen because it causes osteoblastic bone lesions as a result of tumor growth and this will better mimic the clinical conditions. This study is important for us to determine the best time point for the subsequent experiments. Tumors will be allowed to grow for up to 8 weeks. As behavior tests are important to provide qualitative and quantitative information about the degree of pain the animals are experiencing, we would like to assess how well the animals react to these tests before and after injection of tumor cells. Then, we would like to correlate this information to the size and morphology of the tumors as determined by MRI and bone lesions activity at the metastases site as determined by SPECT. In addition, while acquiring MRI images, the imaging protocol will also be optimized to image the tumors in bones. Following euthanization, the tumor-bearing bones and non-tumor bearing bones will be acquired to study the micro-architectural changes in bones and for histology. Blood samples obtained from *vena saphena* (puncture, awake animal) at different time point in this experiment will provide information regarding the immune response as a result of tumor growth and prostate specific antigen (PSA) level. Rise in PSA level is often used in clinic to associate with localized or metastatic prostate cancer. This will give us readout to correlate to the effectiveness of our treatment.

The success of the tumor model establishment will be assessed based on the readout parameters summarized below:

i) behavior tests – assess if sign of pain relief is present
ii) MRI – assess if the tumor size and morphology changes
iii) SPECT– assess if the degree of bone activity changes
iv) Blood samples (obtained from *vena saphena*) – immune response and PSA level
v) Post-mortem – assess micro-architectural changes in bone by micro CT and histology, assess the degree of tissue damaged, especially the periosteum as periosteal denervation is speculated to be one of the main sources of pain relief

For this pilot study, 6 times 3 animals are considered necessary as this is the first time that this model is set up. It is absolutely necessary to practice tumor induction, which is a difficult surgical intervention, to ensure reproducible future experiments. Animals will be ordered in different batches to allow for refinement of technique and reduction in the required number of animals. In addition, animals in this group will be used as controls for Experiment 3. Since this is the first time

that we induce this tumor and the expected difficulties of injecting cells in the osseous we expect a loss of 40%.

Total animals needed for experiment 1 = **18 rats**
18 rats, loss = 40%; (a-0.40*a=18, a= 30) → 30 rats

Experiment 2: Development and optimization of ..... for treatment of bone metastases.

Since this is only technical optimization of the whole protocol used for Experiment 3 we expect to have a coefficient of variation (σ) of 35%. To be able to detect 65% changes (δ) with a power of 80% and a confidence interval of 95%, the minimum number of animal needed for each group based on Sach's formula is: $15.7 * (35/65)^2 = 4.6$ rats → 5 rats.

Part A: Optimization of .....parameters

.....

For each of the 5 parameters, 3 different ..... will be tested. The efficiency of ..... procedures will be assessed based on the readout parameters summarized below:

vi) behavior tests – assess if sign of pain relief is present
vii) MRI – assess if the tumor size and morphology changes
viii) SPECT– assess if the degree of bone activity changes
ix) Blood samples (obtained from *vena saphena*)– immune response and PSA level
x) Post-mortem – assess micro-architectural changes in bone by ..... and histology, assess the degree of tissue damaged, especially the periosteum as periosteal denervation is speculated to be one of the main sources of pain relief

If an optimal protocol ..... is achieved only 5 animals will be treated with it (for generating statistics necessary in Experiment 3) after which Experiment 2 will be immediately ended. Loss of animals due to tumor growth, ..., anesthesia, injection of tracers and/or contrast agents or surgery is estimated to be 15%.

Rats needed per group: 5 rats, loss = 15%; (a-0.15*a=5, a=5.88) → 6 rats
Total rats for Part 2A = 6*5*3 = 90 rats
Of these 90 rats, 30 rats will be healthy instead of tumor bearing and will be moved to animal group 19.

Part B: Optimization of .....
Similar to Part 2A, the same parameters will also need to be optimized for..... Therefore, number of animals required is similar to Part 2A.

Rats needed per group: 5 rats, loss = 15%; (a-0.15*a=5, a=5.88) → 6 rats
Total rats for Part 2A = 6*5*3 = 90 rats
Of these 90 rats, 30 rats will be healthy instead of tumor bearing and will be moved to animal group 20.

Results from this section will be combined with the results of therapeutic study in Experiment 3.
Total animals needed for experiment 2: 90+90 = **180 rats**

Experiment 3: Therapeutic evaluation of ..... palliative treatments.

Based on experience from Experiment 1 and 2, we can now investigate the treatment efficacy of ....., ... to relief pain. The stage of tumor growth used is similar to Experiment 2. The efficiency of ..... will be assessed based on the readout parameters summarized below:

i) behavior tests – assess if sign of pain relief is present
ii) MRI – assess if the tumor size and morphology changes
iii) SPECT– assess if the degree of bone activity changes
iv) Blood samples (obtained from *vena saphena*) – immune response and PSA level
v) Post-mortem – assess micro-architectural changes in bone by ..... and histology, assess the degree of tissue damaged, especially the periosteum as periosteal denervation is speculated to be one of the main sources of pain relief

Similarly, we expect to have a coefficient of variation (σ) of 30%. To be able to detect 40% changes (δ) with a power of 80% and a confidence interval of 95%, the minimum number of animal needed for each group based on Sach's formula is: $15.7 * (30/40)^2 = 8.8$ rats → 9 rats.

Part A: ..... of tumor-bearing bone

Animals will be subjected to ..... based on the procedures optimized in Part 2A and similar efficacy readout. As results will be combined with Experiment 2A, only 4 animals are needed in this group to reach σ of 30% and δ of 40%. Loss of animals due to tumor growth, ..., anesthesia, injection of tracers and contrast agents or surgery is estimated to be 15%.

Rats needed: 4 rats
4 rats, loss = 15%; (a-0.15*a=4, a= 4.7) → 5 rats

Part B: Tumor-bearing bone with .....

This is a control group of part 3A as animals are needed to investigate how effective is ..... compared to ..... animals. As results will be combined with Experiment 1, no animals are needed for this group. Only if we do not have enough results from Experiment 1, animals will be requested for this group. Loss of animals due to tumor growth, anesthesia, injection of tracers and contrast agents or surgery is estimated to be 15%.

Rats needed: 9 rats
9 rats, loss = 15%; (a-0.15*a=9, a= 10.6) → 11 rats

Part C: Sham-operated animals

A group of sham operated animals are needed. This group of animals is needed for us to correlate that the different efficiency readout results that we observed in Part 2A and Experiment 3 are due to the presence of tumor and ..... and not due to the surgery that we performed. Animals will be subjected to sham operation and all the following experimental procedures as Part 2A, with the exception that ..... will be given. Loss of animals due to anesthesia, injection of tracers and/or contrast agents or surgery is estimated to be 10%.

Rats needed: 9 rats
9 rats, loss = 10%; (a-0.10*a=9, a= 10) → 10 rats

Part D: Healthy animals

As a control, a group of healthy animals will be subjected to similar experimental procedure as in Part 2 with the exception of tumor induction, ... and ..... As the efficiency of ..... will be

assessed based on behavior tests, MRI, SPECT, immune response, PSA level, micro-architectural changes in bone and histology, it is important to know how different are all these readouts in healthy animals. To give an example, readouts such as behavior tests and histology are very important because variation in behavior response may be huge in between healthy animals and tissue processing for histology may affect tissue morphology, especially since the periosteum is a very thin layer. Loss of animals due to anesthesia, injection of tracers and/or contrast agents or surgery is estimated to be 10%.

Rats needed: 9 rats
9 rats, loss = 10%; (a-0.10*a=9, a= 10) → 10 rats

Part E: ..... tumor bearing bone
Animals will be subjected to ..... based on the procedures optimized in Part 2A and similar efficacy readout. As results will be combined with Experiment 2A, only 4 animals are needed in this group to reach σ of 30% and δ of 40%. Loss of animals due to tumor growth, ..., anesthesia, injection of tracers and contrast agents or surgery is estimated to be 15%.

Rats needed: 4 rats
4 rats, loss = 15%; (a-0.15*a=4, a= 4.7) → 5 rats

Part F: .....
As shown in literature, ..., thereby exposing the tumor to high concentration of drugs and subsequently, improving the anti-tumor effects. Here, we would like to extend the application to metastatic prostate cancer in bones. ..... will be applied in combination with ..... Loss of animals due to tumor growth, ..., anesthesia, injection of tracers, contrast agents and ..... or surgery is estimated to be 15%.

Rats needed: 9 rats
9 rats, loss = 15%; (a-0.15*a=9, a= 10.6) → 11 rats

Part G: .....
In addition to the chemotherapeutic drug mentioned in Part 3F, therapeutic effect of ..... will also be investigated. Here, the ..... will be used. Loss of animals due to tumor growth, ..., anesthesia, injection of tracers, contrast agents and ..... or surgery is estimated to be 15%.

Rats needed: 9 rats
9 rats, loss = 15%; (a-0.15*a=9, a= 10.6) → 11 rats

Part H: .....
..... Hence, animals in this group serve as the controls for Part 3F and G. We are interested to compare the therapeutic effect of ....., with ..... Loss of animals due to ....., anesthesia, injection of tracers, contrast agents and ..... or surgery is estimated to be 15%.

Rats needed: 9 rats
9 rats, loss = 15%; (a-0.15*a=9, a= 10.6) → 11 rats

Part I: .....
Another clinical approved drug of interest is ..... in Part 3F, G and H. ..... Loss of animals due to ....., anesthesia, injection of tracers, contrast agents and ..... or surgery is estimated to be 15%.

Rats needed: 9 rats
9 rats, loss = 15%; (a-0.15*a=9, a= 10.6) → 11 rats

Part J: Healthy animal ..... (animal groep 17)
As a control to the bone .....tumor carrying limb, a group of animals will be subjected to similar experimental procedures as in Part 2A with the exception of tumor induction. This group of animals is needed for us to correlate if any effect on the bone that was observed is due to ..... or due to a combination of .....and tumor growth. Loss of animals due to anesthesia, injection of tracers and/or contrast agents or surgery is estimated to be 10%.

Rats needed: 9 rats
9 rats, loss = 10%; (a-0.10*a=9, a= 10) → 10 rats

Part K: Tumor bearing animal with analgesic (diergroep 18)
The bone metastases model in this study has both tumor growths in the bone marrow and around the bone. In order to confirm that the pain behavior showed by the animals is due to pain and not due to the size of tumor around the bone (which may affect the motoric function), a group of tumor carrying animals receiving analgesic is required. Loss of animals due to anesthesia, injection of tracers and/or contrast agents or surgery is estimated to be 10%.

Rats needed: 9 rats
9 rats, loss = 10%; (a-0.10*a=9, a= 10) → 10 rats

Total animals needed for experiment 3: 5+11+10+10+5+11+11+11+11+ 10+10 = **105 rats**

Experiment 4: Therapeutic evaluation of ..... to further improve palliative pain management.

In this experiment, we are interested to investigate if ..... will lead to more sustainable pain reduction and subsequently, prolong time to relapse. The ..... tested in Experiment 3 will be used in this experiment.

The efficiency of the treatments will be assessed based on the readout parameters summarized below:

i) behavior tests – assess if sign of pain relief is present
ii) MRI – assess if the tumor size and morphology changes
iii) SPECT– assess if the degree of bone activity changes
iv) Blood samples (obtained from *vena saphena*) – immune response and PSA level
v) Post-mortem – assess micro-architectural changes in bone by ..... and histology, assess the degree of tissue damaged, especially the periosteum as periosteal denervation is speculated to be one of the main sources of pain relief

Part A: .....
Animals in this group are used to investigate whether ..... will lead to more sustainable pain reduction and subsequently, prolong time to relapse. In this study, ... Loss of animals due to ....., anesthesia, injection of tracers, contrast agents and ..... or surgery is estimated to be 15%.

Rats needed: 9 rats
9 rats, loss = 15%; (a-0.15*a=9, a= 10.6) → 11 rats

Part B: .....
Similar to Part 4A, animals in this group are used to investigate whether a..... will lead to more sustainable pain reduction. In this study, ... Loss of animals due to ..... anesthesia, injection of tracers, contrast agents and ..... or surgery is estimated to be 15%.

Rats needed: 9 rats
9 rats, loss = 15%; (a-0.15*a=9, a= 10.6) → 11 rats

Part C: .....
..... However, bioavailability of ..... at tumor site may be less due to faster clearance rate or lower injection dose to limit adverse side effects. We are interested to compare ..... Loss of animals due to ....., anesthesia, injection of tracers, contrast agents and ..... or surgery is estimated to be 15%.

Rats needed: 9 rats
9 rats, loss = 15%; (a-0.15*a=9, a= 10.6) → 11 rats

Part D: .....
Similar to the motivation explained in Part 4C, we are interested to compare ..... Loss of animals due to ....., anesthesia, injection of tracers, contrast agents and d..... or surgery is estimated to be 15%.

Rats needed: 9 rats
9 rats, loss = 15%; (a-0.15*a=9, a= 10.6) → 11 rats

Total animals needed for experiment 4: 11*4= **44 rats**

Dierproef

8. Experiment

In all experiments, animals will be euthanized by the procedure described in ..... under anesthesia immediately at the end of therapeutic assessment specified. When the blood should be removed for further bio-analysis or histology, the euthanization can also be performed by tissue perfusion fixation (.....). Please refer to ..... for behavior tests, ... for intra-osseous injection of tumor cells, ...

For all animals, body weight will be taken for every behavior test up to a maximum of 52 times. Behavior tests will be performed 3 times before tumor cells injection, and 2 times after. Upon onset of pain (uncoordinated movement), behavior tests will be performed 3 times per week. Animals will be handled and fixed regularly (max: everyday) to train the animals to be accustomed to the procedures and to reduce their stress levels before behavior tests.

Experiment 1: Establishing bone metastases tumor model

Part A: Tumor rats

In this experiment, the tumor model will be established in rats by intra-osseous injection of ..... tumor cells. This cell line is chosen because it causes osteoblastic bone lesions as a result of tumor growth and this will better mimic the clinical conditions. This study is important for us to determine the best time point for the subsequent experiments. Tumors will be allowed to grow for up to 8 weeks.

Before the injection of tumor cells, animals are first subjected to behavior tests for 3 days. This is to allow the animals to be accustomed to the behavior tests and as a control to how the animals react to the tests with the absence of tumors. Behavior tests are important because they provide qualitative and quantitative information about the degree of pain the animals are experiencing. For the behavior tests, a total of 3 tests will be performed. In ..... in the appendixes, we have included 4 tests, of which we would prefer the first 3 tests. We would like the DEC committee's opinion and comment if certain tests are preferred. Test 3 and 4 (Hargreaves test and von Frey test) are replaced with the Open-Field Test using the IR Actimeter System. This system allows for locomotor activity and exploration in rodents. Animals are allowed to walk freely in the cage and are tested individually for a maximum of 1 hour per test run.

Following injection of tumor cells, behavior tests will be performed weekly up to a maximum of 8 weeks and then, the animals will be euthanized. In order to correlate tumor growth and bone lesions activity with the degree of pain the animals are experiencing, MRI will be used to assess the size and morphology of the tumors, SPECT will be used to assess bone lesions activity at the metastases site and with CT overlay for anatomical information. MRI and SPECT will only be performed for a maximum of 4 times within the 8 week for tumor and bone characterization. Contrast agent will be injected for MRI and radioactive tracers will be injected for SPECT. In between each MRI and SPECT scans, animals are allowed a minimum of 3 days rest.

200 µL Blood will also be sampled from *vena saphena* (puncture, awake animal) a maximum of 2 times before and a maximum of 2 times every week after the injection of tumor cells. Blood is taken after each behavior test session (*vena saphena* puncture, awake animal) and is used to assess the immune response and prostate specific antigen (PSA) level. Rise in PSA level is often used in clinic to associate with localized or metastatic prostate cancer. This will give us readout to

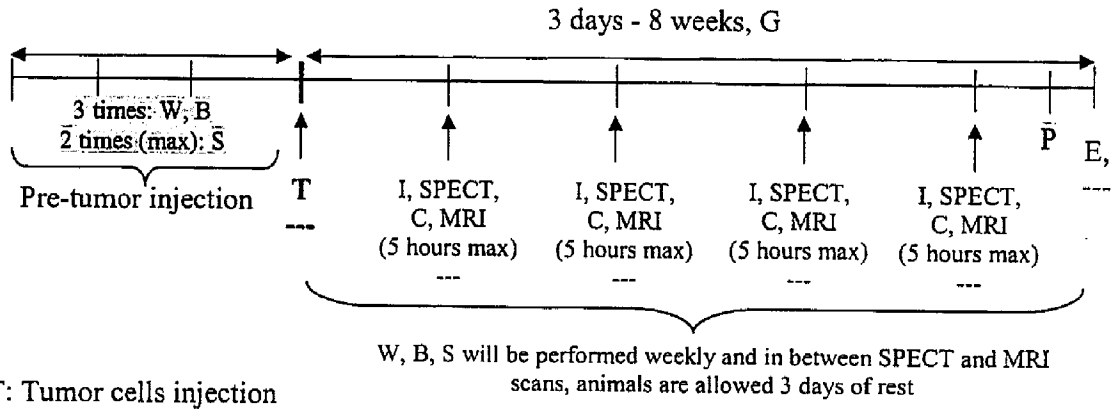
correlate to the effectiveness of our treatment. Within the 8 weeks, after animals are euthanized, the tumor bearing bones and the surrounding muscle tissues are carefully excised and fixed. μCT will be used to analyze micro-architectural changes in bones and histology will be used to assess tissue damage that may be caused by ..... Other organs will also be obtained for histology.

Histology is a very important tool to analyze cellular changes in bone metastases after .....treatments. For example, differentiating necrotic regions resulting from ..... from viable tissues is of great importance. ..... Another histological method is to utilize the enzymatic activity of nicotinamide adenine dinucleotide diaphorase (NADH) in viable cells to reduce the NADH substrates. However, NADH staining method is not applicable for bone metastases analysis as the tissue processing procedures for sectioning of bone requires decalcification for approximately 2 weeks and enzymes present in the bone would have been destroyed.

As a result, we would like to perform *in vivo* staining of cells using pimonidazole. Pimonidazole is a hypoxia marker, which is activated in hypoxic cells to form stable adducts with thiol groups. This staining method has been shown to work in bone and is able to differentiate between viable and necrotic tumor cells.

The pimonidazole probe will be injected at 60 mg/kg body weight to all animals 1-24 hours prior to euthanization. Injection will be performed through either i.p. or i.v. in awake or anesthetized animals. We would like to request for a longer injection time points prior to euthanization (1-24 hours) as we are not sure of the optimum probe uptake duration for our animal model. We will first start with 90 minutes prior to euthanization as stated in literatures and refine our experimental procedures from there.

A schematic representation of the experimental flow is shown below:



T: Tumor cells injection
G: Tumor growth
W: Determine body weight
B: Behavior tests
S: Blood sampling from *vena saphena* (puncture, awake animal)
I: Injection of radioactive tracers (allow 0-4 hours for optimal tracer uptake and injection will be performed without anesthesia)
C: MRI contrast agent injection
E: Euthanized
P: i.v. or i.p. injection of pimonidazole

---: under anesthesia

Experiment 2: Development and optimization of ..... for treatment of bone metastases.

..... Based on our knowledge in experiment 1, we will choose a time point of tumor growth for protocol optimization.

.....

As a result, we would like to perform *in vivo* staining of cells using pimonidazole. Pimonidazole is a hypoxia marker, which is activated in hypoxic cells to form stable adducts with thiol groups. This staining method has been shown to work in bone and is able to differentiate between viable and necrotic tumor cells.

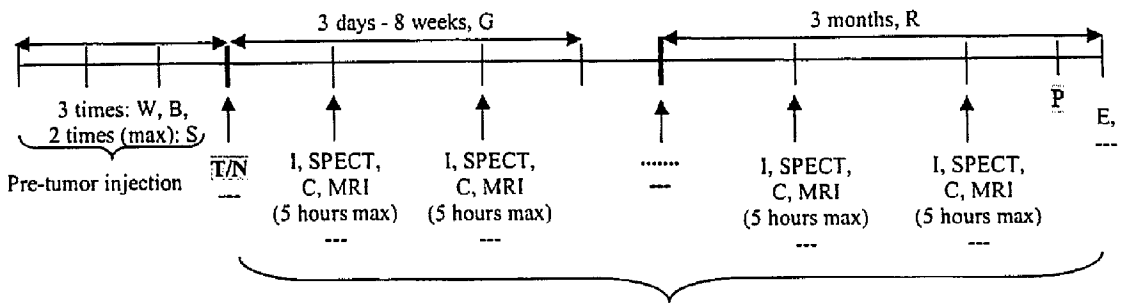
The pimonidazole probe will be injected at 60 mg/kg body weight to all animals 1-24 hours prior to euthanization. Injection will be performed through either i.p. or i.v. in awake or anesthetized animals. We would like to request for a longer injection time points prior to euthanization (1-24 hours) as we are not sure of the optimum probe uptake duration for our animal model. We will first start with 90 minutes prior to euthanization as stated in literatures and refine our experimental procedures from there.

.....

Part A: Optimization of ..... - diergroep 2 (tumor bearing) and diergroep 19 (healthy)

As the protocols optimized in this section will be used for therapeutic studies, the animals will be treated similar to animals in therapeutic study. Initially, all animals will go through the same tumor induction procedure as explain in Experiment 1. The tumor induction procedure will not be performed on healthy animals (diergroep 19). Once the desired tumor stage is reached, animals will be treated with ..... Then, animals are allowed to recover for up to a maximum of 3 months and then, animals are euthanized. During the recovery period, animals will be monitored using the efficiency assessment readouts as discussed in Section 7, Part 2A. MRI and SPECT will be performed a maximum of 5 times from 3 days after the tumor induction until 3 months after the treatment. Contrast agent will be injected for MRI and radioactive tracers will be injected for SPECT. In between each MRI and SPECT scans, animals are allowed a minimum of 3 days rest. 200 µL blood will also be sampled from *vena saphena* (puncture, awake animal) a maximum of 2 times before and a maximum of 2 times every week after the injection of tumor cells. Blood is taken after each behavior test session (*vena saphena* puncture, awake animal) and is used to assess the immune response and prostate specific antigen (PSA) level.

After animals are euthanized, the tumor bearing bones and the surrounding muscle tissues are carefully excised and fixed. ..... changes in bones and histology will be used to assess tissue damage that may be caused ..... Other organs will also be obtained for histology. A schematic representation of the experimental flow is shown below:



W, B, S will be performed weekly and in between SPECT and MRI scans, animals are allowed 3 days of rest

T/N: Tumor cells injection or without for healthy animals
G: Tumor growth
W: Determine body weight
B: Behavior tests
S: Blood sampling from *vena saphena* (puncture, awake animal)
I: Injection of radioactive tracers (allow 0-4 hours for optimal tracer uptake and injection will be performed without anesthesia)
C: MRI contrast agent injection
.....
R: Recovery
P: i.v. or i.p. injection of pimonidazole
E: Euthanized
---: under anesthesia

Part B: Optimization of ..... - diergroep 3 (tumor bearing) and diergroep 20 (healthy)

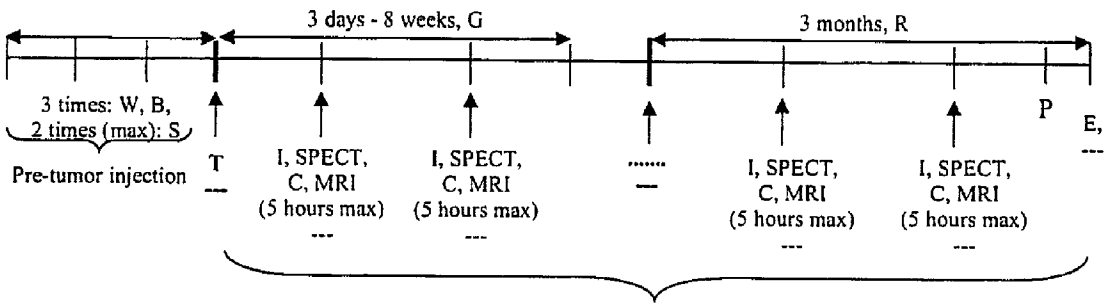
Animals in this group will go through the same procedures as animals in Part 2A. .....

Experiment 3: Therapeutic evaluation of ..... palliative treatments.

Based on experience from Experiment 1 and 2, we can now investigate the treatment efficacy of ..... The stage of tumor growth used is similar to Experiment 2. The efficiency of the treatments will be assessed based on the readout parameters summarized below and in Section 7, Part 2A:

i) behavior tests – assess if sign of pain relief is present
ii) MRI – assess if the tumor size and morphology changes
iii) SPECT– assess if the degree of bone activity changes
iv) Blood samples (obtained from *vena saphena*) – immune response and PSA level
v) Post-mortem – assess micro-architectural changes in bone by ..... and histology, assess the degree of tissue damaged, especially the periosteum as periosteal denervation is speculated to be one of the main sources of pain relief

All animals in this group will go through the same experimental procedures as animals in Part 2. A schematic representation of the experimental flow is shown below:



W, B, S will be performed weekly and in between SPECT and MRI scans, animals are allowed 3 days of rest

T: Tumor cells injection
G: Tumor growth
W: Determine body weight
B: Behavior tests
S: Blood sampling from *vena saphena* (puncture, awake animal)
I: Injection of radioactive tracers (allow 0-4 hours for optimal tracer uptake and injection will be performed without anesthesia)
C: MRI contrast agent injection
.....
R: Recovery
P: i.v. or i.p. injection of pimonidazole
E: Euthanized
---: under anesthesia

Part A: ..... of tumor-bearing bone
Animals will be subjected to the same experimental procedures as Part 2A. Tumor cells will be injected and ..... will be applied.

Part B: Tumor-bearing bone with .....
Animals will be subjected to the same experimental procedures as Part 2A. Tumor cells will be injected and but, no ..... will be applied.

Part C: Sham-operated animals
Animals will be subjected to the same experimental procedures as Part 2A. Tumor cells will not be injected but, medium used to resuspend the tumor cells will be used. .....

Part D: Healthy animals
Animals will be subjected to the same experimental procedures as Part 2A. However, tumor cells will not be injected, ... will not be applied.

Part E: ..... of tumor bearing bone
Animals will be subjected to the same experimental procedures as Part 2A. Tumor cells will be injected and ..... will be applied.

Part F: .....
Animals will be subjected to the same experimental procedures as Part 2A. Tumor cells will be injected, and ..... will be applied in combination with .....

Part G: .....
Animals will be subjected to the same experimental procedures as Part 2A. Tumor cells will be injected, and ..... will be applied in combination with .....

Part H: .....
Animals will be subjected to the same experimental procedures as Part 2A. Tumor cells will be injected, and ..... will be applied in combination with clinically approved .....

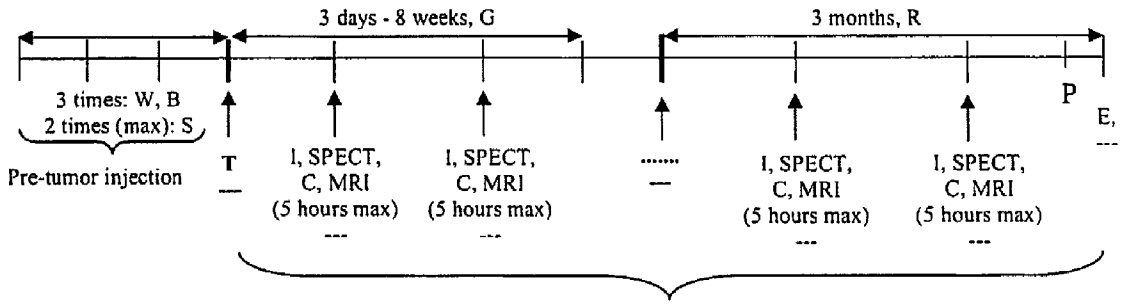
Part I: .....
Animals will be subjected to the same experimental procedures as Part 2A. Tumor cells will be injected, and ..... will be applied in combination with clinically approved .....

Part J: Healthy animal with ..... (diergroep 17)
Animals will be subjected to the same experimental procedures as Part 2A. However, tumor cells will not be injected.

Part K: Tumor bearing animal with analgesic (diergroep 18)
Animals will be subjected to the same experimental procedures as Part 2A. However, ...on these animals. Instead, analgesic, such as Rimadyl, Temgesic, or Meloxicam [14], will be given at least 30 minutes prior to behavior test, when needed, to allow the drug to be metabolized and take effects.

Experiment 4: Therapeutic evaluation of ..... to further improve palliative pain management.

All animals in this group will go through the same experimental procedures as animals in Part 2A. A schematic representation of the experimental flow is shown below:



T: Tumor cells injection
G: Tumor growth
W: Determine body weight
B: Behavior tests
S: Blood sampling from *vena saphena* (puncture, awake animal)

I: Injection of radioactive tracers (allow 0-4 hours for optimal tracer uptake and injection will be performed without anesthesia)
C: MRI contrast agent injection
.....
R: Recovery
P: i.v. or i.p. injection of pimonidazole
E: Euthanized
---: under anesthesia

Part A: .....
Animals will be subjected to the same experimental procedures as Part 2A. Tumor cells will be injected, ... will be applied in combination with ..... will be performed last.

Part B: .....
Animals will be subjected to the same experimental procedures as Part 2A. Tumor cells will be injected, ... will be applied in combination with ..... will be performed last.

Part C: .....
Animals will be subjected to the same experimental procedures as Part 2A. Tumor cells will be injected, ... will be applied in combination with ..... will be performed last.

Part D: .....
Animals will be subjected to the same experimental procedures as Part 2A. Tumor cells will be injected, ... will be applied in combination with ..... will be performed last.

9. Experimentele condities

9a. Anesthesie

The rats will be placed under anesthesia by administration of isoflurane (induction 3-4%, 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 and on surgery table using a face mask.

9b. Pijnbestrijding

In all cases when tumor induction are needed, analgesia will be given 30 minutes before the start of the surgery by administration of Rimadyl = carprofen (subcutaneous injection of 3 mg/kg) or Temgesic = buprenorfine (subcutaneous injection of 0.05 mg/kg). ..... During the observation weeks no pain medication will be given preventing any interference with the behavioral tests.

9c. Euthanasie en Humane eindpunten

- The animals will be euthanized under anesthesia by means of cervical dislocation 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. When the blood should be removed for further bio-analysis or histology, the euthanization can also be performed by tissue perfusion fixation.
- The code of practice in cancer research (Inspectie W&V, 1999) [13] will be used as guideline for responsible endpoints. When a weight loss of more than 15% is observed, behaviour and locomotion of the animal becomes seriously abnormal (from paresis to paralysis), bone fracture, ulcerations, respiratory distress, infections or other serious clinical symptoms are present, the animal will be euthanized prematurely after consultation with the article 12 or 14 officer. Premature euthanization will be discussed with the article 12 or 14 officer when large metastasized tumors are visible on MRI/SPECT or are detected by palpation. The decision to premature euthanization of the animal will be determined by the size and the region of the metastatic tumor.

Zorg

10a. Ongerief

Experiment 1:

Treatment	Repetition	Duration	Code
Anesthesia	6	1-5 hours	04
Surgery for tumor cells injection	1	1-2 hours	03
Tumor growth in bones	1	3 days to 8 weeks	05
Behavior tests	2x/week (max: 19x)	1-2 hours	03
Injection of radioactive tracers	4	15 minutes	03
Injection of contrast agents	4	15 minutes	03
Blood sampling (*vena saphena*)	2x/week (max: 18x)	10 minutes	03
MRI+SPECT	4	5 hours	03
I.p. or i.v. injection of pimonidazole in awake or under anesthesia	1	15 minutes	02
Euthanasia under anesthesia	1	30 minutes	02
		Total:	05

Experiment 2:

Part A: Optimization of ..... (tumor bearing)

Treatment	Repetition	Duration	Code
Anesthesia	7	1-5 hours	04
Surgery for tumor cells injection	1	1-2 hours	03
Tumor growth in bones	1	5 months	05
Behavior tests	2x/week (max: 45x) 3x/week before ..... (max. 2x extra) 7 days after ..... (5x extra) Total: 45+2+5=max. 52	1-2 hours	04
Injection of radioactive tracers	5	15 minutes	03
Injection of contrast agents	5	15 minutes	03
Blood sampling (*vena saphena*)	2x/week (max: 44x)	10 minutes	04
MRI+SPECT	5	5 hours	03
I.p. or i.v. injection of pimonidazole in awake or under anesthesia	1	15 minutes	02
Euthanasia under anesthesia	1	30 minutes	02
.....	1	1 hour	03
		Total:	05

Part A: Optimization of ..... (healthy)

Treatment	Repetition	Duration	Code
Anesthesia	7	1-5 hours	04
Behavior tests	2x/week (max: 45x) 3x/week before ..... (max. 2x extra) 7 days after ..... (5x extra) Total: 45+2+5=max. 52	1-2 hours	04
Injection of radioactive tracers	5	15 minutes	03
Injection of contrast agents	5	15 minutes	03
Blood sampling (*vena saphena*)	2x/week (max: 44x)	10 minutes	04
MRI+SPECT	5	5 hours	03
I.p. or i.v. injection of pimonidazole in awake or under anesthesia	1	15 minutes	02
Euthanasia under anesthesia	1	30 minutes	02
.....	1	1 hour	03
		Total:	04

Part B: Optimization of ..... (tumor bearing)

Treatment	Repetition	Duration	Code
Anesthesia	7	1-5 hours	04
Surgery for tumor cells injection	1	1-2 hours	03
Tumor growth in bones	1	5 months	05
Behavior tests	2x/week (max: 45x) 3x/week before ..... (max. 2x extra) 7 days after ..... (5x extra) Total: 45+2+5=max. 52	1-2 hours	04
Injection of radioactive tracers	5	15 minutes	03
Injection of contrast agents	5	15 minutes	03
Blood sampling (*vena saphena*)	2x/week (max: 44x)	10 minutes	04
MRI+SPECT	5	5 hours	03
I.p. or i.v. injection of pimonidazole in awake or under anesthesia	1	15 minutes	02
Euthanasia under anesthesia	1	30 minutes	02
.....	1	1 hour	03
.....	1	5 minutes	03
		Total:	05

Part B: Optimization of ..... (healthy)

Treatment	Repetition	Duration	Code
Anesthesia	7	1-5 hours	04
Behavior tests	2x/week (max: 45x) 3x/week before ..... (max. 2x extra) 7 days after ..... (5x extra) Total: 45+2+5=max. 52	1-2 hours	04
Injection of radioactive tracers	5	15 minutes	03
Injection of contrast agents	5	15 minutes	03
Blood sampling (*vena saphena*)	2x/week (max: 44x)	10 minutes	04
MRI+SPECT	5	5 hours	03
I.p. or i.v. injection of pimonidazole in awake or under anesthesia	1	15 minutes	02
Euthanasia under anesthesia	1	30 minutes	02
.....	1	1 hour	03
.....	1	5 minutes	03
		Total:	04

Experiment 3:

Part A: ..... of tumor-bearing bone

Treatment	Repetition	Duration	Code
Anesthesia	7	1-5 hours	04
Surgery for tumor cells injection	1	1-2 hours	03
Tumor growth in bones	1	5 months	05
Behavior tests	2x/week (max: 45x) 3x/week before ..... (max. 2x extra) 7 days after ..... (5x extra) Total: 45+2+5=max. 52	1-2 hours	04
Injection of radioactive tracers	5	15 minutes	03
Injection of contrast agents	5	15 minutes	03
Blood sampling (*vena saphena*)	2x/week (max: 44x)	10 minutes	04
MRI+SPECT	5	5 hours	03
I.p. or i.v. injection of pimonidazole in awake or under anesthesia	1	15 minutes	02
Euthanasia under anesthesia	1	30 minutes	02
.....	1	1 hour	03
		Total:	05

Part B: Tumor-bearing bone with no .....

Treatment	Repetition	Duration	Code

Anesthesia	7	1-5 hours	04
Surgery for tumor cells injection	1	1-2 hours	03
Tumor growth in bones	1	5 months	05
Behavior tests	2x/week (max: 45x) 3x/week before ..... (max. 2x extra) 7 days after ..... (5x extra) Total: 45+2+5=max. 52	1-2 hours	04
Injection of radioactive tracers	5	15 minutes	03
Injection of contrast agents	5	15 minutes	03
Blood sampling (*vena saphena*)	2x/week (max: 44x)	10 minutes	04
MRI+SPECT	5	5 hours	03
I.p. or i.v. injection of pimonidazole in awake or under anesthesia	1	15 minutes	02
Euthanasia under anesthesia	1	30 minutes	02
		Total:	05

Part C: Sham-operated animals

Treatment	**Repetition**	**Duration**	**Code**
Anesthesia	7	1-5 hours	04
Surgery for medium injection	1	1-2 hours	03
Behavior tests	2x/week (max: 45x) 3x/week before ..... (max. 2x extra) 7 days after ..... (5x extra) Total: 45+2+5=max. 52	1-2 hours	04
Injection of radioactive tracers	5	15 minutes	03
Injection of contrast agents	5	15 minutes	03
Blood sampling (*vena saphena*)	2x/week (max: 44x)	10 minutes	04
MRI+SPECT	5	5 hours	03
I.p. or i.v. injection of pimonidazole in awake or under anesthesia	1	15 minutes	02
Euthanasia under anesthesia	1	30 minutes	02
		Total:	05

Part D: Healthy animals

Treatment	**Repetition**	**Duration**	**Code**

Anesthesia	6	1-5 hours	04
Behavior tests	2x/week (max: 45x) 3x/week before ..... (max. 2x extra) 7 days after ..... (5x extra) Total: 45+2+5=max. 52	1-2 hours	04
Injection of radioactive tracers	5	15 minutes	03
Injection of contrast agents	5	15 minutes	03
Blood sampling (*vena saphena*)	2x/week (max: 44x)	10 minutes	04
MRI+SPECT	5	5 hours	03
I.p. or i.v. injection of pimonidazole in awake or under anesthesia	1	15 minutes	02
Euthanasia under anesthesia	1	30 minutes	02
		Total:	04

Part E: ..... of tumor-bearing bone

Treatment	**Repetition**	**Duration**	**Code**
Anesthesia	7	1-5 hours	04
Surgery for tumor cells injection	1	1-2 hours	03
Tumor growth in bones	1	5 months	05
Behavior tests	2x/week (max: 45x) 3x/week before ..... (max. 2x extra) 7 days after ..... (5x extra) Total: 45+2+5=max. 52	1-2 hours	04
Injection of radioactive tracers	5	15 minutes	03
Injection of contrast agents	5	15 minutes	03
Blood sampling (*vena saphena*)	2x/week (max: 44x)	10 minutes	04
MRI+SPECT	5	5 hours	03
I.p. or i.v. injection of pimonidazole in awake or under anesthesia	1	15 minutes	02
Euthanasia under anesthesia	1	30 minutes	02
.....	1	1 hour	03
		Total:	05

Part F: .....

Treatment	**Repetition**	**Duration**	**Code**

Anesthesia	7	1-5 hours	04
Surgery for tumor cells injection	1	1-2 hours	03
Tumor growth in bones	1	5 months	05
Behavior tests	2x/week (max: 45x) 3x/week before ..... (max. 2x extra) 7 days after ..... (5x extra) Total: 45+2+5=max. 52	1-2 hours	04
Injection of radioactive tracers	5	15 minutes	03
Injection of contrast agents	5	15 minutes	03
Blood sampling (*vena saphena*)	2x/week (max: 44x)	10 minutes	04
MRI+SPECT	5	5 hours	03
Euthanasia under anesthesia	1	30 minutes	02
.....	1	1 hour	03
.....	1	1 hour	03
I.p. or i.v. injection of pimonidazole in awake or under anesthesia	1	15 minutes	02
		Total:	05

Part G: .....

Treatment	**Repetition**	**Duration**	**Code**
Anesthesia	7	1-5 hours	04
Surgery for tumor cells injection	1	1-2 hours	03
Tumor growth in bones	1	5 months	05
Behavior tests	2x/week (max: 45x) 3x/week before ..... (max. 2x extra) 7 days after ..... (5x extra) Total: 45+2+5=max. 52	1-2 hours	04
Injection of radioactive tracers	5	15 minutes	03
Injection of contrast agents	5	15 minutes	03
Blood sampling (*vena saphena*)	2x/week (max: 44x)	10 minutes	04
MRI+SPECT	5	5 hours	03
Euthanasia under anesthesia	1	30 minutes	02
.....	1	1 hour	03
.....	1	1 hour	03
I.p. or i.v. injection of pimonidazole in awake or under anesthesia	1	15 minutes	02
		Total:	05

Part H: .....

Treatment	**Repetition**	**Duration**	**Code**
Anesthesia	7	1-5 hours	04
Surgery for tumor cells injection	1	1-2 hours	03
Tumor growth in bones	1	5 months	05
Behavior tests	2x/week (max: 45x)	1-2 hours	04

	3x/week before ..... (max. 2x extra) 7 days after ..... (5x extra) Total: 45+2+5=max. 52		
Injection of radioactive tracers	5	15 minutes	03
Injection of contrast agents	5	15 minutes	03
Blood sampling (*vena saphena*)	2x/week (max: 44x)	10 minutes	04
MRI+SPECT	5	5 hours	03
Euthanasia under anesthesia	1	30 minutes	02
.....	1	1 hour	03
.....	1	1 hour	03
I.p. or i.v. injection of pimonidazole in awake or under anesthesia	1	15 minutes	02
		Total:	05

Part I: .....

Treatment	**Repetition**	**Duration**	**Code**
Anesthesia	7	1-5 hours	04
Surgery for tumor cells injection	1	1-2 hours	03
Tumor growth in bones	1	5 months	05
Behavior tests	2x/week (max: 45x) 3x/week before ..... (max. 2x extra) 7 days after ..... (5x extra) Total: 45+2+5=max. 52	1-2 hours	04
Injection of radioactive tracers	5	15 minutes	03
Injection of contrast agents	5	15 minutes	03
Blood sampling (*vena saphena*)	2x/week (max: 44x)	10 minutes	04
MRI+SPECT	5	5 hours	03
Euthanasia under anesthesia	1	30 minutes	02
.....	1	1 hour	03
.....	1	1 hour	03
I.p. or i.v. injection of pimonidazole in awake or under anesthesia	1	15 minutes	02
		Total:	05

Part J: Healthy animal with .....

Treatment	**Repetition**	**Duration**	**Code**
Anesthesia	7	1-5 hours	04
Surgery for tumor cells injection	1	1-2 hours	03
Behavior tests	2x/week (max: 45x) 3x/week before	1-2 hours	04

	(max. 2x extra) 7 days after ..... (5x extra) Total: 45+2+5=max. 52		
Injection of radioactive tracers	5	15 minutes	03
Injection of contrast agents	5	15 minutes	03
Blood sampling (*vena saphena*)	2x/week (max: 44x)	10 minutes	04
MRI+SPECT	5	5 hours	03
Euthanasia under anesthesia	1	30 minutes	02
.....	1	1 hour	03
I.p. or i.v. injection of pimonidazole in awake or under anesthesia	1	15 minutes	02
		Total:	05

Part J: Healthy animal with .....

Treatment	Repetition	Duration	Code
Anesthesia	7	1-5 hours	04
Surgery for tumor cells injection	1	1-2 hours	03
Behavior tests	2x/week (max: 45x) 3x/week before ..... (max. 2x extra) 7 days after ..... (5x extra) Total: 45+2+5=max. 52	1-2 hours	04
Injection of radioactive tracers	5	15 minutes	03
Injection of contrast agents	5	15 minutes	03
Blood sampling (*vena saphena*)	2x/week (max: 44x)	10 minutes	04
MRI+SPECT	5	5 hours	03
Euthanasia under anesthesia	1	30 minutes	02
.....	1	1 hour	03
I.p. or i.v. injection of pimonidazole in awake or under anesthesia	1	15 minutes	02
		Total:	05

Part K: Tumor bearing animal with analgesic

Treatment	Repetition	Duration	Code
Anesthesia	7	1-5 hours	04
Surgery for tumor cells injection	1	1-2 hours	03
Tumor growth in bones	1	5 months	05
Behavior tests	2x/week (max: 45x)	1-2 hours	04

	3x/week upon onset of pain (max. 2x extra) Total: 45+2=max. 47		
Injection of radioactive tracers	5	15 minutes	03
Injection of contrast agents	5	15 minutes	03
Blood sampling (*vena saphena*)	2x/week (max: 44x)	10 minutes	04
MRI+SPECT	5	5 hours	03
Euthanasia under anesthesia	1	30 minutes	02
Injection of analgesic	Before behavior tests when needed (max:47x)	2 minutes	03
I.p. or i.v. injection of pimonidazole in awake or under anesthesia	1	15 minutes	02
		Total:	05

Experiment 4:

Part A: .....

Treatment	**Repetition**	**Duration**	**Code**
Anesthesia	7	1-5 hours	04
Surgery for tumor cells injection	1	1-2 hours	03
Tumor growth in bones	1	5 months	05
Behavior tests	2x/week (max: 45x) 3x/week before ..... (max. 2x extra) 7 days after ..... (5x extra) Total: 45+2+5=max. 52	1-2 hours	04
Injection of radioactive tracers	5	15 minutes	03
Injection of contrast agents	5	15 minutes	03
Blood sampling (*vena saphena*)	2x/week (max: 44x)	10 minutes	04
MRI+SPECT	5	5 hours	03
Euthanasia under anesthesia	1	30 minutes	02
.....	1	1 hour	03
.....	1	1 hour	03
.....	1	1 hour	03
I.p. or i.v. injection of pimonidazole in awake or under anesthesia	1	15 minutes	02
		Total:	05

Part B: .....

Treatment	**Repetition**	**Duration**	**Code**
Anesthesia	7	1-5 hours	04
Surgery for tumor cells injection	1	1-2 hours	03
Tumor growth in bones	1	5 months	05
Behavior tests	2x/week (max: 45x) 3x/week before ..... (max. 2x extra)	1-2 hours	04

	7 days after ..... (5x extra) Total: 45+2+5=max. 52		
Injection of radioactive tracers	5	15 minutes	03
Injection of contrast agents	5	15 minutes	03
Blood sampling (*vena saphena*)	2x/week (max: 44x)	10 minutes	04
MRI+SPECT	5	5 hours	03
Euthanasia under anesthesia	1	30 minutes	02
.....	1	1 hour	03
.....	1	1 hour	03
.....	1	1 hour	03
I.p. or i.v. injection of pimonidazole in awake or under anesthesia	1	15 minutes	02
		Total:	05

Part C: .....

Treatment	**Repetition**	**Duration**	**Code**
Anesthesia	7	1-5 hours	04
Surgery for tumor cells injection	1	1-2 hours	03
Tumor growth in bones	1	5 months	05
Behavior tests	2x/week (max: 45x) 3x/week before ..... (max. 2x extra) 7 days after ..... (5x extra) Total: 45+2+5=max. 52	1-2 hours	04
Injection of radioactive tracers	5	15 minutes	03
Injection of contrast agents	5	15 minutes	03
Blood sampling (*vena saphena*)	2x/week (max: 44x)	10 minutes	04
MRI+SPECT	5	5 hours	03
Euthanasia under anesthesia	1	30 minutes	02
.....	1	1 hour	03
.....	1	1 hour	03
.....	1	1 hour	03
I.p. or i.v. injection of pimonidazole in awake or under anesthesia	1	15 minutes	02
		Total:	05

Part D: .....

Treatment	**Repetition**	**Duration**	**Code**
Anesthesia	7	1-5 hours	04
Surgery for tumor cells injection	1	1-2 hours	03
Tumor growth in bones	1	5 months	05
Behavior tests	2x/week (max: 45x) 3x/week before	1-2 hours	04

	(max. 2x extra) 7 days after ..... (5x extra) Total: 45+2+5=max. 52		
Injection of radioactive tracers	5	15 minutes	03
Injection of contrast agents	5	15 minutes	03
Blood sampling (*vena saphena*)	2x/week (max: 44x)	10 minutes	04
MRI+SPECT	5	5 hours	03
Euthanasia under anesthesia	1	30 minutes	02
.....	1	1 hour	03
.....	1	1 hour	03
.....	1	1 hour	03
I.p. or i.v. injection of pimonidazole in awake or under anesthesia	1	15 minutes	02
		Total:	05

10b. Welzijnsevaluatie

..... The VO 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 officers 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 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

Aan:

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

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

Geachte Onderzoeker,

Uw projectaanvraag: "*Assessment of pain relief of prostate cancer bone metastasis treated with* ", is op de DEC vergadering van 24 juni 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

- De DEC merkt op dat GGO niet van toepassing is op dit DEC protocol (er wordt niet gewerkt met genetisch gemodificeerde dieren) en verzoekt daarom het GGO nummer te verwijderen.
- Bij punt 6 verzoekt de DEC "**The** research proposal" aan te passen in "**This DEC** proposal is internally assessed and approved by the PI".
- Bij punt 7c mist de DEC de relevante uitleesparameter van alle experimenten.
- Bij punt 7c- experiment 2- verzoekt de DEC de uitval per groep in de berekening mee te nemen.
- Bij punt 7c "Part I- Hyperthermia- staat bij het totaal aantal dieren nodig voor experiment 4, dit moet experiment 3 zijn.
- De DEC verzoekt aan te geven hoe de bloedafname plaats vindt.
- De DEC vraagt zich af of 45 gedragstesten en 44 bloedafnames niet ongerief code 04 moet zijn?

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 **2011-094**, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

From:
Sent: maandag 4 juli 2011 17:09
To:
Cc:
Subject: project 2011-094, respons MQ-350-11
Attachments: 20110704_2011-094_Bone metastases model_MQ 350-11.docx

Beste secretaris DEC

In antwoord op de vragen/opmerkingen van de DEC naar aanleiding van projectaanvraag 2011-094:

De DEC heeft een aantal vragen en opmerkingen:

- De DEC merkt op dat GGO niet van toepassing is op dit DEC protocol (er wordt niet gewerkt met genetisch gemodificeerde dieren) en verzoekt daarom het GGO nummer te verwijderen.
  Het GGO nummer is verwijderd (tezamen met een zin in punt 11).

- Bij punt 6 verzoekt de DEC "The research proposal" aan te passen in "**This DEC** proposal is internally assessed and approved by the PI".
  De zin is aangepast.

- Bij punt 7c mist de DEC de relevante uitleesparameter van alle experimenten.
  De relevante uitleesparameters zijn bij alle experimenten toegevoegd.

- Bij punt 7c- experiment 2- verzoekt de DEC de uitval per groep in de berekening mee te nemen.
  De uitval per groep is berekend en de aantallen aangepast.

- Bij punt 7c "Part I- Hyperthermia- staat bij het totaal aantal dieren nodig voor experiment 4, dit moet experiment 3 zijn.
  Dit is aangepast.

- De DEC verzoekt aan te geven hoe de bloedafname plaats vindt.
  Via de vena saphena (wakker dier), dit is in de tekst toegevoegd.

- De DEC vraagt zich af of 45 gedragstesten en 44 bloedafnames niet ongerief code 04 moet zijn?
  De ongeriefcode is aangepast in 04.

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

Met vriendelijke groet

Maastricht University

Faculty of Health, Medicine and Life Sciences

Aan:

Ons kenmerk *Doorkiesnummer* *Maastricht*
13-07-2011

Project: *Assessment of pain relief of prostate cancer bone metastasis treated with MR guided HIFU protocols.*

Verantwoordelijk onderzoeker (VO):

Hierbij 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:	**2011-094**
Diersoort:	**rat**
Aantal dieren:	**339**
Einddatum:	**13-07-2015**

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