Begeleidingsformulier aanvraag dierproef DEC- UM

Versie 2006

Herziene versie

DECNR: 2011-076

Ontvangen: 23-06-2011

DEC datum goedkeuring#	Type aanvraag [2]
23-06-2011	Nieuw

VROM/GGONR[3]
08-047

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	30983016N

Titel van het onderzoek:

Role of microRNA-155 in bleomycin-induced fibrosis

startdatum 01-07-2011 einddatum [9] 01-07-2015 *Duur van de proef [10]: 1 mnd*

	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. Verantwoordelijk medewerker (VM) GGO[7]				Art.9	
4. overige uitvoerenden				Art.9/12[8] Anders[6]:	
5. **PI**				Art.9	

Diergroep	**1**	**2**	**.3**	**4**	**5**	**6**	.
ctrl/exp/sham	control	exp	control	control	exp	exp	
Diersoort	01	01	01	01	01	01	
Stam	C57BL/6	C57BL/6	C57BL/6	C57BL/6	C57BL/6	C57BL/6	
Construct / mutatie ?	nvt	nvt	nvt	miR-155-/-	nvt	miR-155-/-	
Herkomst (leverancier) *	01	01	01	01	01	01	
Aantal	2	12	40	40	40	40	
Geslacht	F	F	F	F	F	F	
Dieren immuuncompetent ?	ja	ja	ja	ja	ja	ja	ja/nee[8]
Leeftijd/gewicht	8w	8w	8w	8w	8w	8w	
Doel van de proef *	33	33	33	33	33	33	
Belang van de proef *	01	01	01	01	01	01	
Toxicologisch onderzoek *	01	01	01	01	01	01	
Bijzondere technieken *	01	05	01	01	05	05	
Anesthesie *	04	04	04	04	04	04	
Pijnbestrijding *	01	01	01	01	01	01	
Mate ongerief *	04	04	04	04	04	04	
Toestand dier einde exp*	01	01	01	01	01	01	

* VHI-coderingen zie bijlage

1 Accountability

Application animal DEC-UM

Title: Role of microRNA- in bleomycin-induced pulmonary fibrosis

1. The purpose of the test.

Pulmonary fibrosis is a chronic progressive disorder in which excessive deposition of extracellular matrix occurs which leads to irreversible scaring of the tissue. This anomaly is one of the main components of different diseases of lung parenchyma like idiopathic pulmonary fibrosis and idiopathic interstitial pneumonia. Also, pronounced fibrosis can be seen in advanced asthma as well as COPD. Current therapies used have been proven to be poorly effective and thus it leads to substantial morbidity and mortality (1).

Pathogenesis of fibrosis is not completely understood and it is considered that multiple factors may play a role but studies have shown that magnitude of fibrosis is tightly regulated by T helper type-1(Th-1) and T helper type-2 (Th-2) cells response. Th-2 cytokines (IL-4 and IL-13) are considered to be the profibrotic while Th-1 cytokine (INF-γ) is generally assumed to be the anti-fibrotic. Among Th-2 cytokines, IL-13 is reported to be the main pro-fibrotic mediator and IL-13 receptor alpha 1 (IL-13Rα1) is the main receptor for IL-13 mediated responses (1,2,3,4).

Interestingly, IL-13Rα1 has been reported to be a direct target of microRNA- miR suggesting that miR- has the ability to regulate IL-13 signalling. Moreover, miR- $CD4^+$ T cells are intrinsically biased toward Th2 differentiation, which may explain the increased airway remodeling seen in the miR- mice Therefore, we hypothesize that miR has an important role in the induction of fibrosis, which will be explored in the present study. For this purpose, fibrosis will be induced in wild type and miR- mice by intratracheal instillation of bleomycin Induction of fibrosis will be quantified at different time points and will be compared between wild type and miR- mice.

Since the role of miRs is increasingly recognized for the regulation of different biological processes, both normal and abnormal, this study will help us to better understand the role of miR- in the pathogenesis of fibrosis which may ultimately be translated in new therapeutic approaches.

2. Social relevance and / or scientific interest

Pulmonary fibrosis is a major component of heterogeneous group of lung parenchymal diseases like idiopathic pulmonary fibrosis, interstitial lung disease, radiation-induced pulmonary fibrosis, scleroderma-induced lung disease and drug-induced lung toxicity which are all associated with substantial morbidity and mortality. For example patients with idiopathic pulmonary fibrosis have median survival of 5 years and objective response rates of less than 30% to conventional treatments (11). Although a lot of work has already been done and is still going on to understand the pathogenesis of pulmonary fibrosis but it is still not completely understood. Also therapeutic strategies in use are poorly effective. So there is a need of better understanding of its pathogenesis and also of development of new therapeutic strategies. In this respect, there's increasing interest for the role of miR in pulmonary fibrosis (8), and in this study we will try to unravel the role of one of these miR's (miR- We expect that this will help us to better understand the pathogenesis of pulmonary fibrosis and ultimate result in the development of new therapies.

3. Alternatives

Studies have shown that pulmonary fibrosis is a very complex disease, which involves different cells at a time and these cells acquire different phenotypes at different stages through cross talk with each other though the production of different cytokines, chemokines and growth factors. Provision of such a natural environment to cell lines or isolated primary cells in in-vitro is fairly impossible. So to understand how really things work under actual conditions, use of animals is indispensable.

4. Ethical consideration

Our lack of understanding of the basic pathogenesis of pulmonary fibrosis may explain the lack of effective treatment strategies. Although corticosteroids and other anti-inflammatory therapies have been used frequently in the past, they have not very successful or even harmful, particularly for idiopathic pulmonary fibrosis (9). So, effective treatments are urgently required and for this a better understanding of disease mechanism is required to find out new therapeutic strategies. We believe that the proposed study will help to improve our knowledge in this regard and since such a mechanistic study is difficult to carry out in humans so we think the use of animals is justified.

5. Scientific Background

Fibrosis is an important pathophysiological hallmark not only in rare pulmonary diseases like idiopathic pulmonary fibrosis, but also in COPD or asthma. Generally, it is accepted that non-genetic fibrotic abnormalities in the lung result from epithelial damage (e.g. caused by cigarette smoke), followed by inflammation and inappropriate remodeling, which may include excessive apoptosis and/or epithelial-mesenchymal transition (EMT) (10,11). Interestingly, increased lung airway remodeling was observed in miRNA- mice suggesting a role for miR- in pulmonary fibrosis. This is supported by several studies demonstrating a role for miR- in EMT (12,13). Moreover, miR- deficient $CD4^+$ T-cells are intrinsically biased towards Th2 differentiation which is strongly linked to fibrogenesis (1). Most important mediators in this process are IL-4 and IL-13, two cytokines which share many functional activities. Regarding pulmonary fibrosis, a dominate role for IL-13 has been shown as over-expression of IL-13 in the lung triggered significant airway fibrosis in the absence of any additional inflammatory stimulus (14). Moreover, collagen deposition was markedly reduced in the lung of mice challenged with bleomycin following treatment with and IL-13 antibody (15). Intriguingly, the IL-13 receptor alpha 1 (IL13Rα1) has recently been shown to be a direct target of miR- indicating that miR- may be able to regulate IL-13 signalling and as such fibrogenesis.

Yet, despite these promising associations and the observed airway remodelling in miR mice, a direct relationship between miR and pulmonary fibrosis has not been established. In this experiment we will study the hypothesis that miR- is an important regulator of inflammation-induced airway remodelling.

6. Wetenschappelijke beoordeling

The current project is internally assessed and approved by the PI of our department

4 Experimental Animals

7. Choice experimental animal

7a. Species, strain / origin / final destination

Specific pathogen free female wild type and miRNA mice on a C57BL/6 background will be used. miRNA- mice are already being maintained at the at Maastricht University, while wild type C57BL/6 will be obtained from Harlan animal research laboratory. At the end of the experiment mice will be sacrificed.

7b. Gender

Gender differences have been reported to play a role in the susceptibility to develop fibrosis in different species and in mice. Regarding bleomycin-induced fibrosis, males seem more sensitive (16). This excludes the use of both males and females as it will increase the number of mice significantly due to the expected variability. The choice for female mice is predominantly based on the assumption that miRNA- mice will be more sensitive than wild type mice. As male mice have been shown to be more sensitive, there is a risk that we may miss the miR- dependent differences because of the already pronounced fibrosis in male mice.

7.c. Number

I) Pilot Study:

Currently, we do not have any experience with the bleomycin model. Moreover, quite a big range of bleomycin dose (1-6U/kg), required to induce fibrosis, is reported in literature, we would like to conduct a small pilot study to find out the optimum dose. For this purpose three different doses are selected, which are 1.5, 3 and 4.5U/kg. As knockout mice are expected to be more susceptible to bleomycine-induced fibrosis, we want to determine the minimum dose required to induce fibrosis in wild type mice because same dose will be used later on in miRNA- mice. So first of all we will use lowest dose of 1.5U/kg in a group of 6 wild type mice. Two mice will be administered intratracheally with 50ul of sterile saline and 4 mice will be likewise instilled with 1.5U/kg of bleomycin diluted in 50ul of sterile saline. Mice will be observed till 21 days and if humane end point (mentioned under 9c) are reached earlier than 21 days, mice will be euthanised. After 21 days both saline- and bleomycin-treated mice will be terminally anesthetized with intra-peritonial injection of pentobarbital (150mg/kg i.p). Lungs will be collected for fibrosis quantitation through histological examination. In case this dose seems insufficient to induce a reasonable amount of fibrosis then higher doses of 3 (and eventually4.5) U/kg will be used in other groups of mice (4 mice for each dose if necessary).

Number of mice:

Control C57BL/6: 2 mice

Bleomycin treated C57BL/6: 4-12 (dependent on the outcome of the dosage finding experiment)

Total number of mice

C57BL/6: 14

II) Main project:

Mice will be divided into four groups

Group-A = experimental group of wild type mice will be treated with saline at time point zero and will be sacrificed at day 7, 14, 21 and 28.

Group-B = experimental group of miRNA- mice will be treated with saline at time point zero and will be sacrificed at day 7, 14, 21 and 28.

Group-C = experimental group of wild type mice will be treated with bleomycin at time point zero and will be sacrificed at day 7, 14, 21 and 28.

Group-D = experimental group of miRNA- mice will be treated with bleomycin at time point zero and will be sacrificed at day 7, 14, 21 and 28

Number of mice

According to the formula of Sachs, the number of mice per group required to achieve a physiologically relevant difference of 20% in fibrosis (assuming a variation of 15%, a p-value <0.05 and a power of 80%) is 8.8. Thus, per group 9 mice are needed. Furthermore, if we assume a dropout rate of 10%, the total number will be 10/group ([a-0,1a]=9; 0,9a=9; a=10).

Total number of mice:
Wild type C57BL/6 : 80 (40 in group A, 40 in group C)
microRNA- knockout mice : 80 (40 in group B, 40 in group D)

6 Animal

8. Experiment

Mice will be anesthetized with isoflurane (3-4%), quickly intubated and bleomycin, diluted in 50ul of sterile saline, will be instilled into trachea. At days 0, 7, 14, 21 and 28 respective number of mice from control group and experimental groups for each time point will be deeply/terminally anesthetized with Sodium Pentobarbital. Lungs and heart will be surgically exposed. To remove the blood in the pulmonary circulation, lungs will be perfused with cold PBS through right ventricle of heart. Broncho-alveolar lavage fluid will be collected by tracheal cannulation. For total RNA isolation, some parts of the lungs will be collected and snap-frozen in liquid nitrogen, while for histopathological examination, other parts lungs will be collected after fixation with 4% phosphate-buffered formaline and will be embedded in paraffin.

9. Experimental conditions

9a. Anesthesia

For intubation and subsequent instilling of bleomycin, isoflurane (3-4%) will be used.

9b. Pain

Since animals will not undergo any surgical procedure the use of a painkiller will not be necessary. However in the earlier phase (3-10 days) after bleomycin instillation pleuritis may develop and animals might show signs of pain. Therefore, animals will be treated with an analgesic (Temgesic, every 12 hours) for 7 days starting at day 3.

9c. Euthanasia and human endpoints

Mice will be euthanized with an overdose of pentobarbital (150mg/kg i.p).

Mice will euthanized earlier than planned in case the animals show severe signs of respiratory distress/clinical signs of pneumonia (open mouthed breathing, pronounced chest movement) or become lethargic and/or display significant weight loss (>10%). Due to development of pulmonary inflammation, during the early phase after the instillation of bleomycin (3-10days), signs of respiratory distress could be more pronounced, so during this period, extra surveillance is necessary.

Care

10a. Inconvenience

Although mice will not undergo any painful surgical procedure, they will have to recover from anesthesia. Moreover, we cannot exclude that bleomycin treatment may cause some discomfort in particular during the first phase (3-10 days after treatment). Also, inflammation will be followed by a fibrogenic phase in which mice may suffer from shortness of breath and weight loss, which might become more severe as fibrosis develops. Based on these expectations, we rate the discomfort score as 04.

10b. Wellness Evaluation

Since we do not have any previous experience of working with bleomycin-induced fibrosis model in mice, so comparison with previous experiments is not possible.

11. Care and housing

Animals will be housed at CPV in groups., under standard housing conditions which are 14h light /10h dark cycle, food and water ad libitum, enriched environment in accordance with daily practice provided by the staff of the CPV, including a standardized humidity, music and day and night rhythm. During the experiment days, it should be considered to check animal's welfare and record their daily status. In case of an emergency, the PI or replacement of the PI needs to be consulted.

12. Expertise

Experiments will be conducted mainly by (Article 9) under direct supervision of has years of experience in animal experimental models.

All procedures (i.p. injection of anesthetic agents, organ collection) will be performed by an "article 9" scientist. Any non qualified person (who has no article 9/12/14 certification) will not participate in any of the procedures or preparations of the laboratory animals.

13. Standard Operation Procedures (SOP)

The procedures on animals are listed as below:

1) intubation of the trachea will be done according to the procedure described in SOP "Far-05-M"

Relevant literature

1. Wynn TA. Fibrotic disease and the T(H)1/T(H)2 paradigm. Nat Rev Immunol 2004;4:583-594.
2. Chiaramonte MG et al. Regulation and function of the interleukin 13 receptor alpha 2 during a T helper cell type 2-dominant immune response. J Exp Med. 2003;197:687-701
3. Wood N, Enhanced interleukin (IL)-13 responses in mice lacking IL-13 receptor alpha 2. J Exp Med. 2003;197:703-9
4. Wilson MS et al. IL-13Ralpha2 and IL-10 co-ordinately suppress airway inflammation, airway-hyperreactivity, and fibrosis in mice. J Clin Invest. 2007;117:2941-51
5. Martinez-Nunez RT et al. The interleukin 13 (IL-13) pathway in human macrophages is modulated by microRNA-155 via direct targeting of interleukin 13 receptor alpha1 (IL13Ralpha1). J Biol Chem 2011;286:1786-94.
6. Rodriguez A et al. Requirement of bic/microRNA-155 for normal immune function. Science. 2007;316(5824):608-11.
7. Adamson IY and Bowden DH. The pathogenesis of bloemycin-induced pulmonary fibrosis in mice. Am J Pathol. 1974;77(2):185-97.
8. Pandit KV et al. MicroRNAs in idiopathic pulmonary fibrosis. Transl Res. 2011; 157(4):191-9
9. Gogali A and Wells AU. New pharmacological strategies for the treatment of pulmonary

fibrosis. Ther Adv Respir Dis. 2010 Dec;4(6):353-66.
10. Crosby LM and Waters CM. Epithelial repair mechanisms in the lung. Am J Physiol Lung Cell Mol Physiol. 2010; 298(6):L715-31.
11. Beers MF and Morrisey EE. The three R's of lung health and disease: repair, remodeling, and regeneration. J Clin Invest. 2011;121(6):2065-73
12. Kong W et al. MicroRNA-155 is regulated by the transforming growth factor beta/Smad pathway and contributes to epithelial cell plasticity by targeting RhoA. Mol Cell Biol. 2008;28(22):6773-84.
13. Pottier N et al. Identification of keratinocyte growth factor as a target of microRNA-155 in lung fibroblasts: implication in epithelial-mesenchymal interactions. PLoS One. 2009 Aug 24;4(8):e6718.
14. Lee CG et al. Interleukin-13 induces tissue fibrosis by selectively stimulating and activating transforming growth factor-β1. J Exp Med. 2001;194(6):809-21.
15. Belperio JA et al. Interaction of IL-13 and C10 in the pathogenesis of bleomycin-induced pulmonary fibrosis. Am J Respir Cell Mol Biol. 2002;27(4):419-27.
16. Voltz JW et al. Male sex hormones exacerbate lung function impairment after bleomycin-induced pulmonary fibrosis. Am J Respir Cel Mol Biol. 2008;39:45-52.

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: 04

Uw referentie: Onze referentie Maastricht, 31-05-2011

Geachte Onderzoeker,

Uw projectaanvraag: "*Role of microRNA- n bleomycin-induced fibrosis*", is op de DEC vergadering van 27 mei 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

- De DEC verzoekt de PI met naam op het voorblad te vermelden.
- Bij punt 5 zou de DEC een schematische toelichting op prijs stellen.
- Bij punt 7c- Main project- De DEC wenst een onderbouwing voor de verschillende tijdstippen (hierbij kan wellicht gebruik gemaakt worden van het schema dat de DEC graag bij punt 5 vermeldt wil zien).
- Bij punt 9c vraagt de DEC zich af hoeveel gewichtsverlies er verwacht wordt bij dit model en of dat een grote uitval met zich mee zou kunnen brengen.
- Punt 9b- De DEC verzoekt alle dieren pijnstilling te geven (in verband met het te verwachten ongerief ten gevolge van pleuritis).
- Bij punt 9c verzoekt de DEC "might be" te vervangen in "is".
- Bij punt 10a mist de DEC het vermelden van het ongerief veroorzaakt door het bijkomen van de anesthesie.

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 **2011-076**, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

Geachte heer leden van de DEC,

Via deze weg willen wij graag reageren op een aantal commentaren van de DEC op project 2011-076.

1. De DEC vraagt om een schematische toelichting bij punt 5. Na herlezing is ons echter duidelijk geworden dat de "scientific background" (punt 5) wel erg gedetailleerd is beschreven. Daarom is besloten om dit onderdeel te herschrijven waarbij een deel achterwege is gelaten i.v.m. de leesbaarheid van het stuk. Ook de punt 1. ("The purpose of the test") is dientengevolge aangepast.

2. De DEC vraagt om de naam van de PI op het voorblad te vermelden. Echter, op het voorblad wordt er nergens specifiek gevraagd om de naam van de PI in te vullen, dus het is mij niet helemaal duidelijk waar dit dient te gebeuren?

3. De DEC vraagt om een onderbouwing voor de verschillende tijdstippen. Deze onderbouwing kan het best geïllustreerd worden door de onderstaande figuur.

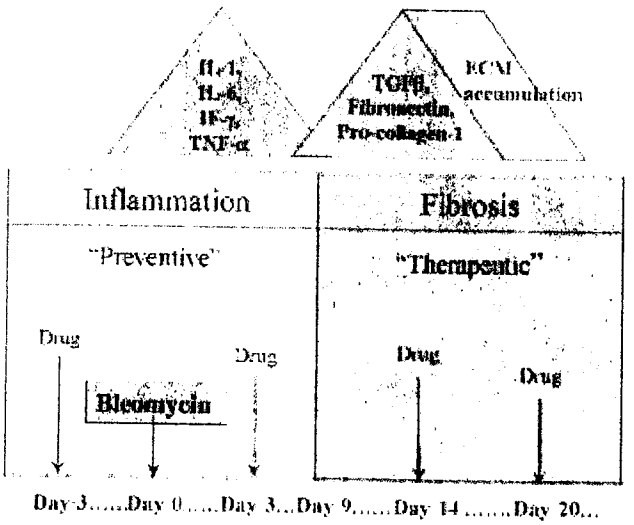

Fig. 1. Sequence of events in bleomycin-induced pulmonary fibrosis. After administration of bleomycin, there is the onset of an acute inflammatory response lasting up to 8 days, followed by fibrogenic changes resulting in deposition of matrix and distortion of lung structure out to 28 or 35 days. Treatments during the first seven days would be considered "preventive" while treatments during the later stages after days 7–10 would be considered "therapeutic". (Moeller al. 2008)

et

Uit deze figuur blijkt duidelijk dat het proces uit meerdere stappen bestaat en dat de voltooiing van het gehele proces tot 35 dagen in beslag neemt. Op basis hiervan zijn de genoemde tijdstippen gekozen.

4. M.b.t. de vraag aangaande het gewichtsverlies; we hebben getracht om een idee te krijgen over het gewichtsverlies aan de hand van gepubliceerde data. Hieruit bleek o.a. dat de resultaten stamafhankelijk zijn. Aangezien wij ze C57Bl/6 stam zullen gebruiken hebben we ons tot deze stam beperkt. Een overzicht van gepubliceerde data vindt u in de onderstaande tabel.

paper	mice	Age/weigth	bleomycin (U/kg)	time	Weigth loss (%)
Gwinn et al (2011)	C57-Fem	8-10w	3,3	8	-7,7
				16	-10,2
Ren et al (2007)	C57-Fem	6-8w	2 (1mg/kg)	7	~ -20,0

				14	~ -15,0
Kim et al (2005)	C57-Male	8-12w	7	7	-26,6
Zu et al (2010)	C57-M	6-8w (20-25g)	6 (3mg/kg)	7 and 14	-18,2
Casey et al (2005)	C57-M/F	25-35g	2 (1mg/kg)	up to 21	no loss

Hieruit blijkt dat het gewichtsverlies zoals verwacht meer uitgesproken is naarmate de muizen jonger/lichter zijn en de dosis hoger is. Wanneer we kijken naar de hoogste dosis, die wij in ons project willen testen (4,5U/kg), dan verwachten we dat het gewichtverlies in normale muizen van 8-12 weken tot zo'n 10% beperkt zal blijven, wat ons inzien acceptabel is.

Wij hopen dat we op deze wijze een aantal van de vragen van de DEC afdoende hebben beantwoord. De overige gevraagde wijzingen zijn rechtstreeks in de aanvraag aangebracht en grijs gemarkeerd. Wij hopen dan ook op een positief advies voor project 2011-076.

Met vriendelijke groet,

From:

Sent: donderdag 23 juni 2011 9:03

To:

Subject: FW: Project 2011-076-w

Attachments: DEC 2011-076 miR in pulmonary fibrosis revised.doc; Reactie op commentaren 2011-076.doc; DEC_voorblad_miR_in_pulmonary_fibrosis[1].doc

Geachte Onderzoeker, beste

De DEC heeft je herziene versie van project 2011-076 bekeken en heeft nog de volgende vraag/opmerking:

- Op het voorblad is plaats genoeg om de PI te vermelden. Ik heb op het voorblad aangegeven waar je dat kunt doen(onder overige uitvoerende), als jij de gegevens nog wilt invullen is dat akkoord.

- De DEC verzoekt je de doseringen bij punt 9b weg te laten en hierover contact op te nemen met de Proefdierdeskundige.
Alleen vermelden dat je temgesic gebruikt is voldoende De doseringen kunnen dan in het werkprotocol vermeld worden.

Wanneer deze 2 kleine aanpassingen gemaakt zijn, kan de aanvraag daarna goedgekeurd worden.

Met vriendelijke groet namens DEC-UM:

Ambtelijk Secretaris Dierexperimentencommissie

Postbus 616-UNS 50-Box 48, 6200 MD Maastricht
T 043 388 1108
E-mail:

Werktijden: Ma-Di-Wo-Don van 08:30 uur tot [illegible]

From:
Sent: dinsdag 21 juni 2011 9:45
To:
Subject: RE: Project 2011-076-w

Beste

Bijgevoegd vind je een aangepaste versie van DEC aanvraag 2011-076. Ook is er een reactie op een aantal vragen van de DEC bijgesloten.

met vriendelijke groet,

Maastricht University

Faculty of Health, Medicine and Life Sciences

Aan:

Ons kenmerk	*Doorkiesnummer*	*Maastricht*
	043-	23-06-2011

Project: *Role of microRNA-155 in bleomycin-induced fibrosis.*

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:	**2011-076**
Diersoort:	**muis**
Aantal dieren:	**174**
Einddatum:	**23-06-2015**

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

Vice-Voorzitter DEC-UM

DEC-UM
Voorzitter DEC-UM
p/a secretariaat DEC-UM

Secretariaat DEC-UM
T (043)

Bezoekadres

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