

|            |  |                                      |                        |             |               |                          |               |               |               |             |
|------------|--|--------------------------------------|------------------------|-------------|---------------|--------------------------|---------------|---------------|---------------|-------------|
|            |  | <b>Inventaris Wob-verzoek W17-11</b> |                        |             |               |                          |               |               |               |             |
|            |  |                                      | <b>wordt verstrekt</b> |             |               | <b>weigeringsgronden</b> |               |               |               |             |
| <b>nr.</b> |  | <b>document NTS 20171674</b>         | <b>reeds openbaar</b>  | <b>niet</b> | <b>geheel</b> | <b>deels</b>             | <b>10.1.c</b> | <b>10.2.e</b> | <b>10.2.g</b> | <b>11.1</b> |
| 1          |  | Origineel aanvraagformulier          |                        |             |               | x                        |               | x             |               |             |
| 2          |  | NTS initieel                         |                        |             | x             |                          |               |               |               |             |
| 3          |  | Projectvoorstel                      |                        |             |               | x                        |               | x             |               |             |
| 4          |  | Bijlage beschrijving dierproeven 1   |                        |             | x             |                          |               |               |               |             |
| 5          |  | Bijlage beschrijving dierproeven 2   |                        |             | x             |                          |               |               |               |             |
| 6          |  | DEC-advies                           |                        |             |               | x                        |               | x             |               |             |
| 7          |  | Ontvangstbevestiging                 |                        |             |               | x                        |               | x             |               |             |
| 8          |  | Verzoek om aanvullende informatie    |                        |             |               | x                        |               | x             |               |             |
| 9          |  | NTS aangepast                        | x                      |             |               |                          |               |               |               |             |
| 10         |  | Adviesnota CCD                       |                        | x           |               |                          |               |               |               | x           |
| 11         |  | Beschikking en vergunning            |                        |             |               | x                        |               | x             |               |             |

1030020171674

1.



## Aanvraag Projectvergunning Dierproeven Administratieve gegevens

- U bent van plan om één of meerdere dierproeven uit te voeren.
- Met dit formulier vraagt u een vergunning aan voor het project dat u wilt uitvoeren. Of u geeft aan wat u in het vergunde project wilt wijzigen.
- Meer informatie over de voorwaarden vindt u op de website [www.zbo-ccd.nl](http://www.zbo-ccd.nl) of in de toelichting op de website.
- Of bel met 0900-2800028 (10 ct/min).

### 1 Gegevens aanvrager

|                                                     |                                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                                                    |                                |                                            |                                                                       |                              |                                 |                 |          |                    |                                       |                |            |  |             |            |  |
|-----------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------|--------------------------------------------|-----------------------------------------------------------------------|------------------------------|---------------------------------|-----------------|----------|--------------------|---------------------------------------|----------------|------------|--|-------------|------------|--|
| 1.1                                                 | Heeft u een deelnemernummer van de NVWA?<br><i>Neem voor meer informatie over het verkrijgen van een deelnemernummer contact op met de NVWA.</i>                          | <input checked="" type="checkbox"/> Ja > Vul uw deelnemernummer in 10300<br><input type="checkbox"/> Nee > U kunt geen aanvraag doen                                                                                                                                                                                                                                                                               |                                |                                            |                                                                       |                              |                                 |                 |          |                    |                                       |                |            |  |             |            |  |
| 1.2                                                 | Vul de gegevens in van de instellingsvergunninghouder die de projectvergunning aanvraagt.                                                                                 | <table><tr><td>Naam instelling of organisatie</td><td>Stichting Katholieke Universiteit Nijmegen</td></tr><tr><td>Naam van de portefeuillehouder of diens gemachtigde</td><td>Instantie voor dierenwelzijn</td></tr><tr><td>KvK-nummer</td><td>4 1 0 5 5 6 2 9</td></tr></table>                                                                                                                                   | Naam instelling of organisatie | Stichting Katholieke Universiteit Nijmegen | Naam van de portefeuillehouder of diens gemachtigde                   | Instantie voor dierenwelzijn | KvK-nummer                      | 4 1 0 5 5 6 2 9 |          |                    |                                       |                |            |  |             |            |  |
| Naam instelling of organisatie                      | Stichting Katholieke Universiteit Nijmegen                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                    |                                |                                            |                                                                       |                              |                                 |                 |          |                    |                                       |                |            |  |             |            |  |
| Naam van de portefeuillehouder of diens gemachtigde | Instantie voor dierenwelzijn                                                                                                                                              |                                                                                                                                                                                                                                                                                                                                                                                                                    |                                |                                            |                                                                       |                              |                                 |                 |          |                    |                                       |                |            |  |             |            |  |
| KvK-nummer                                          | 4 1 0 5 5 6 2 9                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                                                    |                                |                                            |                                                                       |                              |                                 |                 |          |                    |                                       |                |            |  |             |            |  |
| 1.3                                                 | Vul de gegevens van het postadres in.<br><i>Alle correspondentie van de CCD gaat naar de portefeuillehouder of diens gemachtigde en de verantwoordelijke onderzoeker.</i> | <table><tr><td>Straat en huisnummer</td><td>[Redacted]</td></tr><tr><td>Postbus</td><td>[Redacted]</td></tr><tr><td>Postcode en plaats</td><td>[Redacted]</td></tr><tr><td>IBAN</td><td>NL90ABNA0231209983</td></tr><tr><td>Tenaamstelling van het rekeningnummer</td><td>UMC St Radboud</td></tr></table>                                                                                                         | Straat en huisnummer           | [Redacted]                                 | Postbus                                                               | [Redacted]                   | Postcode en plaats              | [Redacted]      | IBAN     | NL90ABNA0231209983 | Tenaamstelling van het rekeningnummer | UMC St Radboud |            |  |             |            |  |
| Straat en huisnummer                                | [Redacted]                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                    |                                |                                            |                                                                       |                              |                                 |                 |          |                    |                                       |                |            |  |             |            |  |
| Postbus                                             | [Redacted]                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                    |                                |                                            |                                                                       |                              |                                 |                 |          |                    |                                       |                |            |  |             |            |  |
| Postcode en plaats                                  | [Redacted]                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                    |                                |                                            |                                                                       |                              |                                 |                 |          |                    |                                       |                |            |  |             |            |  |
| IBAN                                                | NL90ABNA0231209983                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                    |                                |                                            |                                                                       |                              |                                 |                 |          |                    |                                       |                |            |  |             |            |  |
| Tenaamstelling van het rekeningnummer               | UMC St Radboud                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                    |                                |                                            |                                                                       |                              |                                 |                 |          |                    |                                       |                |            |  |             |            |  |
| 1.4                                                 | Vul de gegevens in van de verantwoordelijke onderzoeker.                                                                                                                  | <table><tr><td>(Titel) Naam en voorletters</td><td>[Redacted]</td><td><input checked="" type="checkbox"/> Dhr. <input type="checkbox"/> Mw.</td></tr><tr><td>Functie</td><td>Universitair docent/Onderzoeker</td><td></td></tr><tr><td>Afdeling</td><td>[Redacted]</td><td></td></tr><tr><td>Telefoonnummer</td><td>[Redacted]</td><td></td></tr><tr><td>E-mailadres</td><td>[Redacted]</td><td></td></tr></table> | (Titel) Naam en voorletters    | [Redacted]                                 | <input checked="" type="checkbox"/> Dhr. <input type="checkbox"/> Mw. | Functie                      | Universitair docent/Onderzoeker |                 | Afdeling | [Redacted]         |                                       | Telefoonnummer | [Redacted] |  | E-mailadres | [Redacted] |  |
| (Titel) Naam en voorletters                         | [Redacted]                                                                                                                                                                | <input checked="" type="checkbox"/> Dhr. <input type="checkbox"/> Mw.                                                                                                                                                                                                                                                                                                                                              |                                |                                            |                                                                       |                              |                                 |                 |          |                    |                                       |                |            |  |             |            |  |
| Functie                                             | Universitair docent/Onderzoeker                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                                                    |                                |                                            |                                                                       |                              |                                 |                 |          |                    |                                       |                |            |  |             |            |  |
| Afdeling                                            | [Redacted]                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                    |                                |                                            |                                                                       |                              |                                 |                 |          |                    |                                       |                |            |  |             |            |  |
| Telefoonnummer                                      | [Redacted]                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                    |                                |                                            |                                                                       |                              |                                 |                 |          |                    |                                       |                |            |  |             |            |  |
| E-mailadres                                         | [Redacted]                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                    |                                |                                            |                                                                       |                              |                                 |                 |          |                    |                                       |                |            |  |             |            |  |
| 1.5                                                 | (Optioneel) Vul hier de gegevens in van de plaatsvervangende verantwoordelijke onderzoeker.                                                                               | <table><tr><td>(Titel) Naam en voorletters</td><td></td><td><input type="checkbox"/> Dhr. <input type="checkbox"/> Mw.</td></tr><tr><td>Functie</td><td></td><td></td></tr><tr><td>Afdeling</td><td></td><td></td></tr><tr><td>Telefoonnummer</td><td></td><td></td></tr><tr><td>E-mailadres</td><td></td><td></td></tr></table>                                                                                   | (Titel) Naam en voorletters    |                                            | <input type="checkbox"/> Dhr. <input type="checkbox"/> Mw.            | Functie                      |                                 |                 | Afdeling |                    |                                       | Telefoonnummer |            |  | E-mailadres |            |  |
| (Titel) Naam en voorletters                         |                                                                                                                                                                           | <input type="checkbox"/> Dhr. <input type="checkbox"/> Mw.                                                                                                                                                                                                                                                                                                                                                         |                                |                                            |                                                                       |                              |                                 |                 |          |                    |                                       |                |            |  |             |            |  |
| Functie                                             |                                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                                                    |                                |                                            |                                                                       |                              |                                 |                 |          |                    |                                       |                |            |  |             |            |  |
| Afdeling                                            |                                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                                                    |                                |                                            |                                                                       |                              |                                 |                 |          |                    |                                       |                |            |  |             |            |  |
| Telefoonnummer                                      |                                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                                                    |                                |                                            |                                                                       |                              |                                 |                 |          |                    |                                       |                |            |  |             |            |  |
| E-mailadres                                         |                                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                                                    |                                |                                            |                                                                       |                              |                                 |                 |          |                    |                                       |                |            |  |             |            |  |

- 1.6 (Optioneel) Vul hier de gegevens in van de persoon die er verantwoordelijk voor is dat de uitvoering van het project in overeenstemming is met de projectvergunning.

|                             |                             |                                                            |
|-----------------------------|-----------------------------|------------------------------------------------------------|
| (Titel) Naam en voorletters | [Redacted]                  | <input type="checkbox"/> Dhr. <input type="checkbox"/> Mw. |
| Functie                     | Instantievoor Dierenwelzijn |                                                            |
| Afdeling                    | [Redacted]                  |                                                            |
| Telefoonnummer              | [Redacted]                  |                                                            |
| E-mailadres                 | [Redacted]                  |                                                            |

- 1.7 Is er voor deze projectaanvraag een gemachtigde?

☒ Ja > Stuur dan het ingevulde formulier *Melding Machtiging* mee met deze aanvraag

☐ Nee

## 2 Over uw aanvraag

- 2.1 Wat voor aanvraag doet u?

☒ Nieuwe aanvraag > Ga verder met vraag 3

☐ Wijziging op (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn  
Vul uw vergunde projectnummer in en ga verder met vraag 2.2

☐ Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn  
Vul uw vergunde projectnummer in en ga verder met vraag 2.3

- 2.2 Is dit een *wijziging* voor een project of dierproef waar al een vergunning voor verleend is?

☐ Ja > Beantwoord dan in het projectplan en de niet-technische samenvatting alleen de vragen waarop de wijziging betrekking heeft en onderteken het aanvraagformulier

☐ Nee > Ga verder met vraag 3

- 2.3 Is dit een *melding* voor een project of dierproef waar al een vergunning voor is verleend?

☐ Nee > Ga verder met vraag 3

☐ Ja > Geef hier onder een toelichting en ga verder met vraag 6

## 3 Over uw project

- 3.1 Wat is de geplande start- en einddatum van het project?

|            |                     |
|------------|---------------------|
| Startdatum | 0 1 . 0 8 . 2 0 1 7 |
| Einddatum  | 0 1 . 0 8 . 2 0 2 2 |

- 3.2 Wat is de titel van het project?

Resolving the cell-specific actions of SDF-1 in kidney injury and regeneration

- 3.3 Wat is de titel van de niet-technische samenvatting?

Onderzoek naar nieuwe aanknopingspunten voor de ontwikkeling van behandelingen etc

- 3.4 Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?

|             |            |
|-------------|------------|
| Naam DEC    | RU DEC     |
| Postadres   | [Redacted] |
| E-mailadres | [Redacted] |

## 4 Betaalgegevens

- 4.1 Om welk type aanvraag gaat het? ☒ Nieuwe aanvraag Projectvergunning € 1.287,00 Lege  
☐ Wijziging € Lege
- 4.2 Op welke wijze wilt u dit bedrag aan de CCD voldoen.  
*Bij een eenmalige incasso geeft u toestemming aan de CCD om eenmalig het bij 4.1 genoemde bedrag af te schrijven van het bij 1.2 opgegeven rekeningnummer.*  
☐ Via een eenmalige incasso  
☒ Na ontvangst van de factuur

## 5 Checklist bijlagen

- 5.1 Welke bijlagen stuurt u mee?
- Verplicht
- ☒ Projectvoorstel
- ☒ Niet-technische samenvatting
- Overige bijlagen, indien van toepassing
- ☐ Melding Machtiging
- ☒ DEC-advies en factuurinformatie

## 6 Ondertekening

- 6.1 Print het formulier uit, onderteken het en stuur het inclusief bijlagen via de beveiligde e-mailverbinding naar de CCD of per post naar:

Centrale Commissie  
 Dierproeven  
 Postbus 20401  
 2500 EK Den Haag

Ondertekening door de instellingsvergunninghouder of gemachtigde (zie 1.6). De ondergetekende verklaart:

- dat het projectvoorstel is afgestemd met de Instantie voor Dierenwelzijn.
- dat de personen die verantwoordelijk zijn voor de opzet van het project en de dierproef, de personen die de dieren verzorgen en/of doden en de personen die de dierproeven verrichten voldoen aan de wettelijke eisen gesteld aan deskundigheid en bekwaamheid.
- dat de dieren worden gehuisvest en verzorgd op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van richtlijn 2010/63/EU, behalve in het voorkomende geval de in onderdeel F van de bijlage bij het bij de aanvraag gevoegde projectvoorstel gemotiveerde uitzonderingen.
- dat door het ondertekenen van dit formulier de verplichting wordt aangegaan de leges te betalen voor de behandeling van de aanvraag.
- dat het formulier volledig en naar waarheid is ingevuld.

|              |                              |
|--------------|------------------------------|
| Naam         |                              |
| Functie      | Instantie voor dierenwelzijn |
| Plaats       | Nijmegen                     |
| Datum        | 08 - 05 - 2017               |
| Handtekening |                              |

**Format****Niet-technische samenvatting**

- Dit format gebruikt u om uw niet-technische samenvatting te schrijven.
- Meer informatie over de niet-technische samenvatting vindt u op de website [www.zbo-ccd.nl](http://www.zbo-ccd.nl).
- Of neem telefonisch contact op. (0900-2800028).

## 1 Algemene gegevens

|     |                          |                                                                                                                          |
|-----|--------------------------|--------------------------------------------------------------------------------------------------------------------------|
| 1.1 | Titel van het project    | Onderzoek naar nieuwe aanknopingspunten voor de ontwikkeling van behandelingen die nierschade kunnen beperken of genezen |
| 1.2 | Looptijd van het project | 1-8-2017 - 1-8-2022                                                                                                      |
| 1.3 | Trefwoorden (maximaal 5) | parietale epitheelcel, nierziekte, proximale tubulus, regeneratie, stromal derived factor 1                              |

## 2 Categorie van het project

2.1 In welke categorie valt het project.

U kunt meerdere mogelijkheden kiezen.

- ☒ Fundamenteel onderzoek
- ☐ Translationeel of toegepast onderzoek
- ☐ Wettelijk vereist onderzoek of routinematige productie
- ☐ Onderzoek ter bescherming van het milieu in het belang van de gezondheid of het welzijn van mens of dier
- ☐ Onderzoek gericht op het behoud van de diersoort
- ☐ Hoger onderwijs of opleiding
- ☐ Forensisch onderzoek
- ☐ Instandhouding van kolonies van genetisch gemodificeerde dieren, niet gebruikt in andere dierproeven

### 3 Projectbeschrijving

|     |                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|-----|-------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 3.1 | Beschrijf de doelstellingen van het project (bv de wetenschappelijke vraagstelling of het wetenschappelijk en/of maatschappelijke belang) | Voor veel patiënten met acute of chronische nierziekten is geen adequate behandeling mogelijk, mede omdat voor verschillende nierziekten de onderliggende mechanismen onbekend zijn. Zowel in acute- als chronische nierschade zijn de nierbuisjes en de kleine bloedvaatjes (glomeruli en microvasculatuur) in de nier beschadigd. Wanneer deze nierschade niet tijdig wordt hersteld/gerepareerd treedt er verlies op van de nierfunctie. SDF-1 is een eiwit dat van belang lijkt te zijn voor een goede conditie en mogelijk herstel van de nierbuisjes en kleine bloedvaatjes. Om dit in detail te onderzoeken zullen we gebruik maken van speciale muizen waarin we SDF-1 of zijn receptoren gericht (cel-specifiek) kunnen uitschakelen. Door in deze muizen nierschade te induceren wordt precies duidelijk waar in de nier en wanneer tijdens het ziekteproces SDF-1 en zijn receptoren hun rol spelen en waar en wanneer er aanknopingspunten zijn voor nieuwe behandelingen. |
| 3.2 | Welke opbrengsten worden van dit project verwacht en hoe dragen deze bij aan het wetenschappelijke en/of maatschappelijke belang?         | Het beschreven onderzoek zal resulteren in nieuwe inzichten in de moleculaire mechanismen die betrokken zijn bij schade en herstel van de nierbuisjes en de kleine bloedvaatjes van de nier. De resultaten van dit onderzoek zullen we gebruiken om een follow-up studie in diersystemen te ontwikkelen. In deze diersystemen voor nierschade willen wij verder bestuderen of behandelingen gericht op SDF-1 de nierschade kunnen voorkomen of herstellen. Uiteindelijk doel is de ontwikkeling van een behandeling die de nierschade remt en herstel van het nierweefsel bevordert.                                                                                                                                                                                                                                                                                                                                                                                                   |
| 3.3 | Welke diersoorten en geschatte aantallen zullen worden gebruikt?                                                                          | De experimenten zullen worden uitgevoerd in muizen. We verwachten gedurende vijf jaar in totaal maximaal 1086 muizen nodig te hebben.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| 3.4 | Wat zijn bij dit project de verwachte negatieve gevolgen voor het welzijn van de proefdieren?                                             | De mogelijke negatieve gevolgen voor de dieren bestaan uit: <ul style="list-style-type: none"><li>- Stress en ongemak door huisvesting in een metabole kooi</li><li>- Stress, ongemak en pijn veroorzaakt door een injectie</li><li>- Ongemak, en conditieverlies veroorzaakt door de nierschade</li><li>- Ongemak en bijwerkingen veroorzaakt door gen-deficiëntie</li><li>- Ongemak veroorzaakt door operatie (ischemie reperfusie model)</li><li>- Ongemak door plaatselijke afbraak van spierweefsel</li></ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |

|     |                                                                             |                                                                                                                                                                                                                |
|-----|-----------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 3.5 | Hoe worden de dierproeven in het project ingedeeld naar de verwachte ernst? | Het merendeel van de dieren in het onderzoek zal licht ongerief ondervinden (~58%). Het overige deel van de dieren zal matig ongerief ondervinden (~42%). Geen van de muizen zal ernstig ongerief ondervinden. |
| 3.6 | Wat is de bestemming van de dieren na afloop?                               | De proefdieren worden aan het einde van het experiment gedood om verder ongerief te voorkomen en om nieren en bloed te kunnen verwijderen voor verdere analyse.                                                |

## 4 Drie V's

|     |                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|-----|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 4.1 | <b>Vervanging</b> Geef aan waarom het gebruik van dieren nodig is voor de beschreven doelstelling en waarom proefdiervrije alternatieven niet gebruikt kunnen worden.          | De rol van SDF-1 in de cel onderzoeken we voor een groot deel in celweek systemen. Echter om de rol van SDF-1 in de (patho)fysiologie van de nier te onderzoeken hebben we een complex organisme nodig met een bloedsomloop en waarin alle verschillende niercellen aanwezig zijn. We kunnen daarom geen gebruik maken van proefdiervrije alternatieven.                                                                                                                                                                                                                                                                                                                                                     |
| 4.2 | <b>Vermindering</b> Leg uit hoe kan worden verzekerd dat een zo gering mogelijk aantal dieren wordt gebruikt.                                                                  | Informatie uit voorgaande studies wordt gebruikt om de dierexperimenten zo te ontwerpen dat er zo min mogelijk dieren worden gebruikt om wetenschappelijk betrouwbare resultaten te krijgen. In de proefopzet zijn 'go/no go' momenten ingebouwd om, op basis van al verkregen resultaten, te beoordelen of de resterende proeven uitgevoerd kunnen/moeten worden. Op die manier wordt het aantal benodigde dieren beperkt tot het minimale aantal.                                                                                                                                                                                                                                                          |
| 4.3 | <b>Verfijning</b> Verklaar de keuze voor de diersoort(en). Verklaar waarom de gekozen diertype model(len) de meest verfijnde zijn, gelet op de doelstellingen van het project. | Alleen in muizen kunnen we het te onderzoeken gen in nierweefsel uitschakelen en de complexe pathologie van de nierziekten van de mens nabootsen. In onze studies maken we gebruik van cel-specifieke induceerbare knock-out muizen. Dit betekent dat we het te onderzoeken gen in specifieke cellen en op elk gewenst moment kunnen uitschakelen. De muis is hierdoor alleen deficiënt voor het gen gedurende het dierexperiment, en niet voorafgaand aan het experiment zoals in een conventionele knock-out muis. Mogelijk ongerief als gevolg van de deficiëntie is naar verwachting ook minder omdat het gen alleen in de te onderzoeken cellen wordt uitgeschakeld en niet in alle cellen van de muis. |

4.4

Vermeld welke algemene maatregelen genomen worden om de negatieve (schadelijke) gevolgen voor het welzijn van de proefdieren zo beperkt mogelijk te houden.

Alle dieren zullen dagelijks gecontroleerd worden op welzijn, waarbij gelet wordt op

- ademhaling,
- toestand van de vacht
- (ernstige) gewichtsafname
- hun gedrag.

Zodra afwijkende zaken worden geconstateerd die het doel van de proef onmogelijk maken of onnodig ongerief veroorzaken, zullen de dieren uit de proef genomen worden.

Sommige muizen zullen gedurende de proeven in een metabole kooi gehuisvest worden om urine (voor analyse) te kunnen opvangen. Om ongerief als gevolg van verblijf in de metabole kooi te beperken zullen er schuilhuisjes in de metabole kooi geplaatst worden.

## 5 In te vullen door de CCD

Publicatie datum

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Beoordeling achteraf

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### Form Project proposal

- This form should be used to write the project proposal of animal procedures.
- The appendix 'description animal procedures' is an appendix to this form. For each type of animal procedure, a separate appendix 'description animal procedures' should be enclosed
- For more information on the project proposal, see our website([www.zbo-ccd.nl](http://www.zbo-ccd.nl)).
- Or contact us by phone (0900-2800028).

## 1 General information

|     |                                                                                              |                                                                                                   |
|-----|----------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|
| 1.1 | Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'. | 10300                                                                                             |
| 1.2 | Provide the name of the licenced establishment.                                              | Stichting Katholieke Universiteit Nijmegen                                                        |
| 1.3 | Provide the title of the project.                                                            | Resolving the cell-specific actions of Stromal Derived Factor 1 in kidney injury and regeneration |

## 2 Categories

|     |                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                             |
|-----|-----------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2.1 | Please tick each of the following boxes that applies to your project. | <input checked="" type="checkbox"/> Basic Research<br><input type="checkbox"/> Translational or applied research<br><input type="checkbox"/> Regulatory use of routine production<br><input type="checkbox"/> Research into environmental protection in the interest of human or animal health or welfare dier<br><input type="checkbox"/> Research aimed at preserving the species subjected to procedures |
|-----|-----------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

|                                                                                                                     |
|---------------------------------------------------------------------------------------------------------------------|
| <input type="checkbox"/> Higher education or training                                                               |
| <input type="checkbox"/> Forensic enquiries                                                                         |
| <input type="checkbox"/> Maintenance of colonies of genetically altered animals not used in other animal procedures |

## 3 General description of the project

### 3.1 Background

Describe the project (motivation, background and context) with respect to the categories selected in 2.

- For legally required animal procedures, indicate which statutory or regulatory requirements apply (with respect to the intended use and market authorisation).
- For routine production, describe what will be produced and for which uses.
- For higher education or training, explain why this project is part of the educational program and describe the learning targets.

Kidney diseases are a dominant problem and also a common complication in critically ill patients. The morbidity and mortality as well as the financial burden to the society still remain unacceptable high [1-3]. The kidney can be affected seriously for several reasons such as sepsis, toxic injury or ischemic insults leading to a rapid failure of the kidney to filter blood, regulate the ion and water balance and generate urine which can have systemically consequences [4]. During kidney diseases, the kidney function declines in response to injury because of a rapid fall in glomerular filtration rate, with a high risk of progression into chronic kidney disease (CKD) and a complete loss of kidney function. After kidney injury, the damaged cells of the different nephron segments have to be repaired by regeneration to restore the kidney function. Given the complex renal structure and multiple cell types present in the kidney, it is likely that different mechanisms of cell renewal and regeneration exist in the kidney. Because of the high incidence and a limitation of available therapies to enhance repair and regeneration after injury, it is of high importance to find pathways which can be pharmacologically triggered to stimulate regeneration or inhibit pathways contributing to a poorer pathologic outcome in kidney disease.

#### ***Chronic kidney disease***

Chronic kidney disease (CKD) is a general term for heterogeneous disorders affecting kidney structure and function. Chronic kidney disease contributes to cardiovascular morbidity and mortality, and often progresses to end stage renal disease. CKD is generally associated with increasing age, diabetes, hypertension, obesity, urological and cardiovascular disease and specific kidney diseases. The increased prevalence of CKD, the high morbidity and mortality rates, the high costs and poor outcomes of treatment constitute a worldwide public health threat.

Regardless of the initial insult(s), CKD is characterized by stereotypical kidney injury responses seen pathologically as glomerulosclerosis, i.e. scarring of the glomerulus (the filtration unit of the kidney), tubular atrophy, peritubular capillary rarefaction as well as interstitial fibrosis and inflammation (5). The fibrosis/sclerosis and capillary rarefaction occur commonly in parallel. On the one hand, microvascular loss may occur as a result of tubular atrophy and fibrosis, whereas on the other hand microvascular loss may itself contribute to kidney fibrosis due to hypoxic injury (6-10). Preservation of the glomerular and peritubular capillary architecture is therefore a central target of both existing and novel renoprotective therapies (9). Recently, the stromal derived factor 1 (SDF-1)/CXCR4/CXCR7-axis has emerged as a major player in renal vascular development, maintenance and neo-angiogenesis (10-12).

### ***The SDF-1/CXCR4/CXCR7-axis***

We will study the SDF-1/CXCR4/CXCR7-axis as an important signalling pathway and possible therapeutic target to treat CKD. In adults, the role of SDF-1 was originally defined in maintenance of the hematopoietic stem cell niche and B-cell lymphopoiesis. However, the near ubiquitous tissue distribution of SDF-1 and its rapid degradation in blood suggests much broader intra-organ specific functions. SDF-1 is a small cytokine belonging to the chemokine family that is also designated chemokine (C-X-C motif) ligand 12 (CXCL12). SDF-1 signals via its main receptor CXCR4. The fundamental nature of the SDF-1/CXCR4 relationship is revealed by the development of identical defects in vasculogenesis and organogenesis leading to lethality in the absence of either gene (13). Recently, CXCR7 has been identified as a second receptor for SDF-1, which acts as a scavenger for SDF-1 (14-16). More recently, SDF-1 was identified as an angiogenic factor, important for the recruitment of endothelial progenitor cells and proliferation of endothelial cells (17-19). Within the kidneys, it has been shown that SDF-1 signalling via its receptor CXCR4 is essential for the development of the kidney vasculature (11;12). Because similar developmental processes may be recapitulated in the disease setting, SDF-1 may have also an important role in the maintenance and regeneration of the capillaries in chronic kidney disease (CKD) (10).

However, the role of SDF-1 signalling in the adult kidney appears to be context-dependent. For instance, several studies in acute kidney injury (AKI) and CKD support a renoprotective function for SDF-1 signalling (10;20;21), whereas other studies have proposed that SDF-1/CXCR4 signalling is associated with progression of kidney fibrosis and inflammation (22-24). In this case, inhibition of the SDF-1/CXCR4 axis resulted in attenuation of the disease (22;24-26). For instance, CXCR4 mediated hyperproliferation of glomerular parietal cells (PECs) may contribute to the development of glomerulosclerosis (22). In addition, persistent expression of SDF-1 by myofibroblast facilitates chronic inflammation by recruitment and retention of leukocytes (27). Therefore, the effects of SDF-1 signalling are likely contextual and varying according to stage of the disease and underlying injurious insult.

### ***SDF-1 signalling in glomerulosclerosis***

Progressive glomerular injury results in glomerulosclerosis, the leading cause for end-stage renal disease. Glomerulosclerosis is characterized by progressive scarring of the glomerulus. In experimental models and human biopsies studies we have shown that activated parietal epithelial cells (PECs) play a crucial role in development of glomerulosclerosis (28-33). A normal glomerulus consists of the endocapillary compartment and epithelial cells: podocytes lining the capillary tuft and PECs lining the inside of Bowman's capsule. Formation of a sclerotic lesion is triggered by a primary insult to podocytes followed by activation of PECs. Migration and proliferation of the activated PECs on the glomerular tuft lead to progression of glomerulosclerosis as more of the former capillary loops are covered by PECs and matrix. Currently, there are limited options for treatment of glomerulosclerosis available. Our findings suggest that persistent activation of PECs is driving disease progression and that pharmacological inactivation of PECs represents an effective and targeted treatment option to slow-down loss of renal function (28;30).

The molecular signalling pathways directing the activation, proliferation and migration of PECs are unknown. The SDF-1/CXCR4 and CXCR7 -axis is a promising candidate for several reasons: In the healthy glomerulus the chemokine SDF-1 is constitutively expressed and interacts presumably in a paracrine fashion with CXCR4 that is almost exclusively expressed by the endothelial cells (11).

In glomerulosclerosis, PECs start to express SDF-1 and show an increased expression of CXCR4 (34). In addition, increased expression of CXCR4 by PECs was shown to be functionally associated with proliferation (22). Furthermore, we showed SDF-1 stimulated chemotaxis of PECs (unpublished observations).

We hypothesize that upregulation of the SDF-1/CXCR4/CXCR7-axis in parietal epithelial cells (PECs) is essential for the activation of PECs and therefore for development of glomerulosclerosis. Thus, Inhibition of SDF-1 or the receptors may attenuate development of glomerulosclerosis.

### ***SDF-1 signalling in tubular injury***

The role of SDF-1 and CXCR4 and CXCR7 may not be restricted to glomerulosclerosis. Tubular cell damage is a common finding in biopsies of patients with renal disease. Progressive glomerular and tubular injury may lead to tubular atrophy and capillary rarefaction and finally fibrosis of the kidney.

Necrosis of the proximal tubular epithelial cells with detachment from the basement membrane as well as the rapid loss of the proximal tubular brush border and the formation of tubular casts due to tubular debris results in a loss of cellular polarity and loss of cell function because proximal tubule cells require attachment to the basement membrane to be totally integrated into the tubule. This loss of cell function is one of the major key players in the pathogenesis of renal functional failure and has to be restored to maintain kidney function. It is widely known that the proximal tubule has the capacity to undergo regeneration after acute kidney injury. But what mechanism or cell drives repair and regeneration of the proximal tubule after injury?

Tubular cell injury is followed by a strong upregulation of SDF-1, CXCR4 and CXCR7 expression in proximal tubular cells (12-14). SDF-1 signalling presumably promotes cell survival and regeneration since partial inhibition of SDF-1 has shown to hinder regeneration of proximal tubular cells (9). Both, glomerular disease and tubular damage are accompanied by loss of functional capillaries and local hypoxia. Hypoxia causes increased expression of several proteins including SDF-1 and its receptors (11, 15), which have the potential to promote neovascularization (16).

We hypothesize that upregulation of SDF-1 and its receptors is essential for proximal tubule regeneration and plays an important role in promoting neovascularization.

Based on the literature and the hypotheses outlined above, it is clear that inhibition of SDF-1 signalling may have advantageous and disadvantageous effects. Nevertheless, the SDF-1/CXCR4/CXCR7-axis represents a promising therapeutic target which requires more detailed studies. Most importantly, the cell-specific and disease stage-dependent effects need to be clarified. In the current proposal we use state of the art conditional knock-out mouse models that allow the study of SDF-1 mediated effects in a cell-specific and time dependent manner.

The studies proposed in this project were described in scientific grant proposals that have been awarded with the prestigious Vidi award from the Talent Scheme of The Netherlands Organisation for Scientific Research (NWO) and a grant from the Dutch Kidney foundation.

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### 3.2 Purpose

Describe the project's main objective and explain why this objective is achievable.

- If the project is focussed on one or more research objectives, which research questions should be addressed during this project?
- If the main objective is not a research objective, which specific need(s) does this project respond to?

Our major goal is to define the intrinsic pathways and signals of kidney epithelial cells that regulate the response and phenotypic changes facilitating cell survival and repair of the kidney epithelial cells and its microvasculature during an episode of kidney injury. In this proposal we will study the importance of SDF-1 signaling in the processes of kidney disease and repair. Ultimately the gained knowledge will be used to identify therapeutic targets that can be used to intervene in the pathological processes or to stimulate kidney regeneration.

More specifically, within this proposal we address important questions within four key objectives which are described in detail in 3.4.2. basic outline of the research strategy:

Key objective 1: What is the expression of SDF-1/CXCR4/CXCR7 in the kidney and in human and experimental kidney disease?

Key objective 2: Is SDF-1 a key mediator of parietal epithelial cell activation and associated proliferation and matrix production in glomerulosclerosis? If so by which receptor (CXCR4 or CXCR7) are the observed effects mediated?

Key objective 3: Is SDF-1 essential for tubular epithelial cell survival and regeneration in tubular injury? If so by which receptor (CXCR4 or CXCR7) are the observed effects in tubular injury mediated?

Key objective 4: Is SDF-1 essential for the integrity of the kidneys microvasculature and does prolonged expression of SDF-1 lead to increased inflammation of the kidney or progression of kidney fibrosis?

We think that our aims are innovative and feasible. The application of genetically engineered mouse models, i.e. podocyte- and tubular cell- specific inducible knock-out mice is cutting-edge technology. By applying these different highly sophisticated mouse models we can unravel the role of SDF-1 signaling in glomerulosclerosis, tubular injury/regeneration as well as in maintenance and regeneration of the vascular structures. The conditional KO mouse lines are described in detail in 3.4.1. Research strategies.

### 3.3 Relevance

What is the scientific and/or social relevance of the objectives described above?

#### ***Societal relevance***

World-wide millions of people are affected by renal diseases, in particular acute kidney injury (AKI) and chronic kidney injury (CKD) which often progresses to End-Stage Renal Disease (ESRD). In the Netherlands, 10% of the Dutch population has chronic kidney injury (1.7 million people). However, 40% of them (680,000 people) are not aware of this injury as complaints often only arise when the renal function has declined to 30%. People with chronic kidney injury have an increased risk of kidney failure, and death from cardiovascular disease. More than 60,000 people have severe kidney failure, and approximately 16,000 of these need renal replacement therapy. There is an unmet need for specific treatment modalities to prevent or treat AKI and CKD, in order to reduce the number of patients with ESRD. More knowledge on the molecular processes involved in the aetiology and progression of renal damage is essential in order to identify specific targets for treatment.

Glomerulosclerosis, scarring of the glomerulus, is a hallmark lesion in CKD and is the leading cause for progression towards the final stage of CKD, ESRD. There are many different underlying diseases that may lead to glomerulosclerosis. The molecular pathways involved in kidney damage and progressive glomerulosclerosis are currently still undefined. Treatment of patients with glomerulosclerosis is therefore mainly supportive (reduction in blood pressure and proteinuria) and not focused on a specific underlying cause or molecular process.

Recent findings by our group and others showed that independent of the underlying aetiology, glomerulosclerosis is always associated with scar formation by PECs. Thus, the pro-fibrotic activation of PECs represents a common principal mechanism in glomerulosclerosis. Identifying the underlying molecular mechanisms could lead to identification of novel effective and highly specific therapeutic approaches.

The results of this project might be used to attenuate glomerulosclerosis by targeting the PECs specifically.

Chronic kidney disease is also a common clinical problem characterized by injured tubular cells and microvascular damage. When tubular and vascular damage is not properly repaired, it may lead to renal fibrosis (interstitial tubular fibrosis/tubular atrophy) and loss of renal function.

Currently, it remains unclear how the kidney regenerates from such injuries and why this sometimes fails and results in chronic renal failure. Understanding of the mechanisms that contribute to the maintenance and/or regeneration of tubular cells and the renal microvasculature may lead to the development of new therapeutic strategies, which will be beneficial for many patients.

## ***Scientific relevance***

The project will reveal knowledge of the regulators of kidney epithelial and endothelial cell regeneration:

Ultimately, the ones to benefit from our research are hopefully patients with kidney disease. In order to lead to improved patient care, our research should reach the following target groups:

- Scientific community: This target group can be best informed through publications in high impact journals and presentations at international congresses (Kidney Week of the American Society of Nephrology, World Congress of Nephrology, Dutch and German annual Nephrology conferences).
- Clinicians treating kidney disease patients: As we are working at an academic hospital that combines high level patient care with top biomedical research and also as a member of the Renal Disorder theme of the Radboud institute of Molecular Life Sciences (RIMLS), we are perfectly positioned to enable the translation of research results in terms relevant to clinicians and (as this proposal exemplifies) the other way around: to translate pressing clinical questions in innovative research solutions. The translation of our research to the clinic will benefit from the direct collaboration in this project with expert nephrologists in The Netherlands and in Germany.

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### **3.4 Research Strategy**

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#### **3.4.1 Provide an overview of the overall design of the project (strategy).**

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Epithelial cells of the kidney are like epithelial cells of the liver and the pancreas, self-renewing at a low rate compared to the epithelial cells in the skin or intestine. However, the quiescent epithelial cells of the kidney are activated during the regenerative response following injury. This activation of the epithelial cells is associated with the activation of a transcriptional program leading to phenotypic changes and dedifferentiation of the epithelial cells facilitating their survival, migration and proliferation. It is a prerequisite and our aim to define and understand the intrinsic and extrinsic signal(s) of epithelial cell regeneration after kidney injury.

As described in the background description of this proposal there are strong indications, based on findings described in the literature and preliminary studies in human biopsies and cell culture, for an important role of the SDF-1/CXCR4/CXCR7 axis in kidney regeneration. To investigate the role of

the SDF-1/CXCR4/CXCR7 axis, we will study the cell specific effects at different time points (e.g. early disease and late advanced disease). The significance of a particular molecule can be tested in knock-out (KO) mice. However SDF-1 and CXCR4 knock-out (KO) mice are lethal. Therefore, functional testing of the importance of the SDF-1/CXCR4/CXCR7-axis in KO mice was only possible during embryogenesis and early organ development. Therefore, the precise role of the SDF-1/CXCR4/CXCR7-axis in the (patho)physiology of injury in fully developed tissues/organs including the kidney, remains largely unknown.

In the current project we will use a recently generated transgenic mouse in which the SDF-1 gene was interposed between two LoxP sites (SDF-1fl/fl), which permits deletion of the SDF-1 gene in mice expressing Cre recombinase (38). To delete SDF-1 expression specifically in either PECs, podocytes or proximal tubule cells, we will crossbreed the SDF-1fl/fl mice with respective mice that express Cre exclusively in the epithelial cells of the nephron (PAX8-rtTA), or podocytes (Pod-rtTA/LC1). The Cre expression is conditional and is induced by doxycycline (DOX) (Tet-On system) or tamoxifen. These cell-specific knock-out models will also be used for knockout of the responsible receptors CXCR4 and CXCR7.

Using the outlined mice, we will for the first time be able to study the cell specific functions of SDF-1 in a time-dependent (disease stage dependent) manner in the (patho)physiology of fully developed kidneys and we will be able to determine if pharmacological targeting of this pathway will be important for better disease outcome in patients with kidney diseases.

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#### 3.4.2 Provide a basic outline of the different components of the project and the type(s) of animal procedures that will be performed.

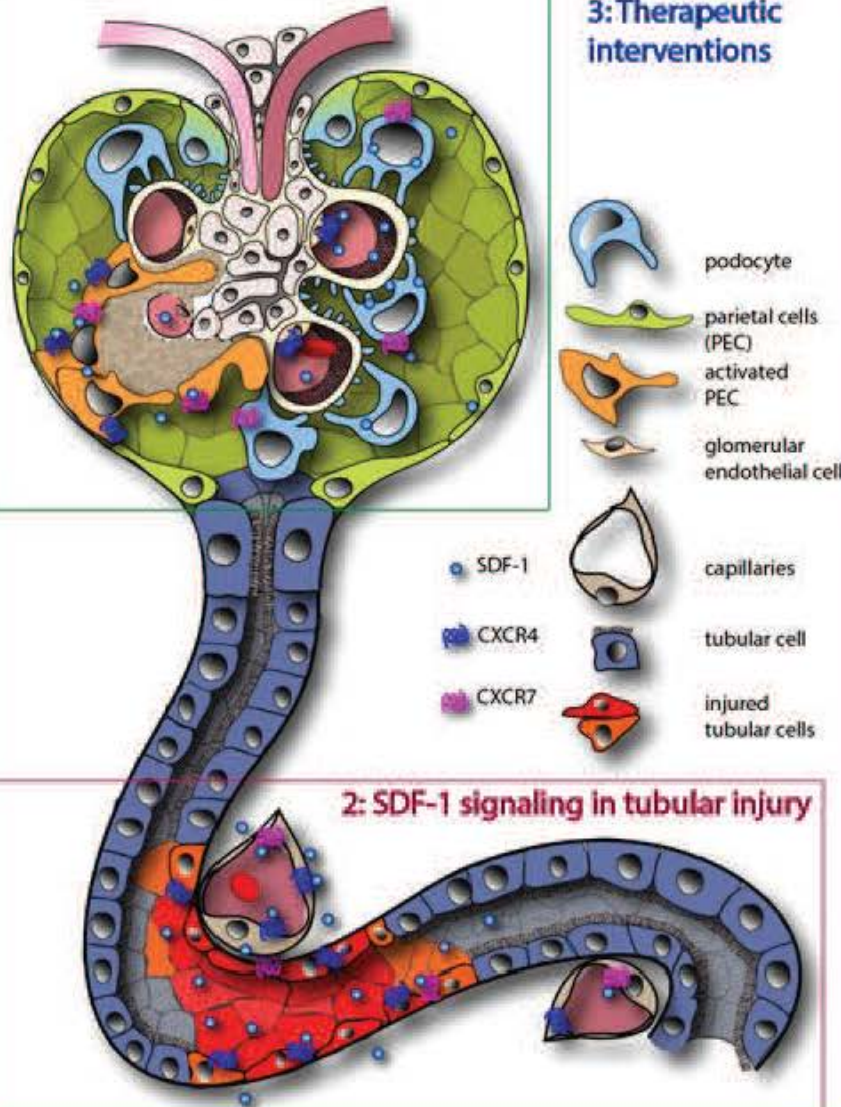
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To enhance our understanding of the role of the SDF-1 pathway in health and disease, we first want to evaluate the expression of SDF-1/CXCR4/CXCR7 in human and experimental renal disease (Key objective 1). Because of the complexity of the nephron, which is the functional unit of the kidney and also because of the great cell heterogeneity, it is of most importance to unravel the effect of the SDF-1 pathway during kidney injury for the different nephron segments. As SDF-1 could have different effects on disease progression and regeneration after renal injury dependent on the underlying disease as outlined in detail in the background description of this project proposal, we want to study the effects of SDF-1/CXCR4/CXCR7 knock-out during experimental glomerular injury (Key objective 2) as well as tubular injury (Key objective 3). It is known that progressive glomerular and tubular injury may lead to tubular atrophy and capillary rarefaction and finally fibrosis of the kidney. Because of this, all kidney tissue from the animal experiments will be analysed for inflammation, fibrosis and microvascular integrity (Key objective 4). In figure 1, an overview of a nephron is given, regarding the different pathological mechanisms inside the glomerulus (1: SDF-1 signalling in glomerulosclerosis, Key objective 1, 2 and) and the tubular system (2: SDF-1 signalling in tubular injury, Key objective 1, 3 and 4). Unravelling the role of SDF-1 and its receptors during these renal pathologies will provide us with important information to detect possible therapeutic treatments (e.g. activation or inhibition of the SDF-1 pathway) which could be tested in a follow-up study (3. Therapeutic interventions).



### 1: SDF-1 signaling in glomerulosclerosis

### 3: Therapeutic interventions



### 2: SDF-1 signaling in tubular injury

Schematic illustration and background information about SDF-1 signaling that will be studied in the two different animal procedures. **1: SDF-1 signaling in the glomerulus** and **2: SDF-1 signaling in tubular injury**. Results from these experiments will help us to create possible follow up studies: **3: Therapeutic interventions**.

**1: SDF-1 signaling in the glomerulus (Key objective 1, 2 and 4)** In healthy glomeruli SDF-1 is expressed by the podocytes and proposed to interact in a paracrine fashion with the CXCR4 positive glomerular endothelial cells and autocrine with the CXCR7 receptor on the podocytes. We hypothesize that this signaling is important for the glomerular integrity, function and regeneration after injury. In glomerulosclerosis activated PECs show an upregulated expression of the receptors. The increased signaling within these PECs may induce PEC activation/proliferation and or migration and subsequent sclerosis of the glomerulus.

**2: SDF-1 signaling in tubular injury (Key objectives 1, 3 and 4)** In tubular damage the injured tubular cells show an increased expression of SDF-1, CXCR4 and CXCR7. It has been proposed that SDF-1 mediated signaling is important for tubular cell survival and regeneration of the tubular cells but also the juxtaposed capillaries. On the other hand increased and prolonged SDF-1 signaling may lead to increased inflammation and fibrosis of the kidney.

**3. Therapeutic interventions (possible follow-up study)** Inhibition of SDF-1 or the receptors may attenuate development of glomerulosclerosis and kidney inflammation and fibrosis. Stimulation of the signaling may stimulate the regeneration of tubuli and kidney vasculature. We believe that intervention in SDF-1 signaling is dependent on the type of injury (glomerular/tubular) and on the stage of the disease.

**Key objective 1. Evaluate the expression of SDF-1/CXCR4/CXCR7 in human and experimental renal disease (animal procedures 1 and 2).**

The expression of the SDF-1/CXCR4/CXCR7-axis components will be analysed at the protein level using immunohistochemistry on kidney sections of patients with acute and progressive chronic renal failure. For comparison we will use renal tissues from 10 normal human kidneys. To identify the best mouse model to study SDF-1 signalling, the human data will be compared to the expression of SDF-1, CXCR4, and CXCR7 that is examined in experimental animal models of glomerulosclerosis (animal procedure 1): (i) anti-Thy-1.1 mouse model (ii) Adriamycin model, and in models of tubular injury (animal procedure 2) (iii) Ischemia reperfusion (I/R) model and (iv) glycerol model. All four models are established models of CKD and develop kidney fibrosis and inflammation. We have experience with all four models. In addition, we will study the expression of SDF-1 in SDF-1-DsRed reporter mice, which will allow sensitive analysis of expression of SDF-1 in time and in location.

These experiments will provide us with the important information to find out which animal models of glomerular and tubular disease resembles best the human situation in regard to SDF-1 signalling. In addition, this experiment will provide information at which time point in disease SDF-1 expression is altered in response to the underlying disease (see flow schema 1).

Dependent on these findings, the best suitable models with the greatest effect on SDF-1 (and receptor CXCR4 and CXCR7) expression during experimental disease will be chosen to continue with Key objective 2 and Key objective 3 **(GO/NO GO decision)**. Furthermore, the best time points for knock-out of the SDF-1/CXCR4/CXCR7 axis during glomerulosclerosis and tubular injury will be determined. This reduces the amount of groups needed for the experiments regarding Key objective 2 and Key objective 3.

**Flow schema 1: Schematic illustration of Key objective 1:**

**Key Objective 1:**

Determine the expression of SDF-1 and its receptors during experimental disease



Thy1.1. model



Adriamycin model



Glycerol model



I/R model

1. Choose animal model

2. Determine exact time points of  
changes in SDF-1/CXCR4/CXCR7  
expression during disease



**Key Objective 2:**

Evaluate the role of the  
SDF-1/CXCR4/CXCR7 axis  
in glomerulosclerosis



**Key Objective 3:**

Evaluate the role of the  
SDF-1/CXCR4/CXCR7  
axis in tubular injury

## **Key objective 2. Evaluate the role of SDF-1/CXCR4/CXCR7-axis in glomerulosclerosis (animal procedure 1).**

To study the role of SDF-1 in the development of glomerulosclerosis, we will generate a podocyte specific SDF-1 conditional knock-out (KO) mice, which allows SDF-1 knock-out upon doxycycline treatment. Established colonies of podocyte-rtTA/LC1 transgenic mice are available and present in the animal facility of the Radboudumc and have been used successfully in our previous studies. First of all the expression of SDF-1 after doxycycline treatment to knock-out SDF-1 will be analysed and compared to wild type mice. Of note, only if the expression of SDF-1 is reduced significantly within the glomeruli of the kidneys the mice will be suitable for further studies **(GO/NO GO Decision)**.

In these mice, glomerulosclerosis will be induced and SDF-1 expression will be inactivated shortly before it becomes upregulated in disease. The exact time point has been established under Key objective 1. Non-transgenic littermates and/or animals that do not receive doxycycline to induce cell-specific gene inactivation will serve as controls for the experiments. This allows us to answer if SDF-1 is a key mediator of parietal epithelial cell activation and associated proliferation and matrix production in glomerulosclerosis. Only if significant effects of SDF-1 knock-out during experimental glomerulosclerosis are observed, the same procedures will be performed with CXCR4 and CXCR7 conditional KO mice to determine which receptor pathway is responsible for the observed effects **(GO/NO GO-Decision)**

To test which receptor pathways mediate the effects of SDF-1 in the development of glomerulosclerosis, mice in which the PECs or podocytes are deficient for CXCR4 or CXCR7 will be analysed. These mice are crossbreds of the PAX8-Cre mice or Pod-rtTA mice and the CXCR4fl/fl and/or CXCR7fl/fl mice. This will lead to deletion of the CXCR4 and/or CXCR7 gene in all epithelial cells of the nephron including the PECs. The efficiency of the knock out will be verified by real time PCR and Western blot analysis of isolated PECs as well as histological analysis. Also in these mice development of glomerulosclerosis will be induced. Littermates lacking the Cre transgene will serve as controls for the experiment. This will allow us to answer via which receptor (CXCR4 or CXCR7) the observed effects in experimental glomerulosclerosis are mediated. The experiments of this key objective are shortly summarized in flow schema 2.

### **Flow schema 2: Schematic illustration of Key objective 2:**

## Key Objective 2:

Evaluate the role of the SDF-1/CXCR4/CXCR7 axis in glomerulosclerosis



### 1. Validation of SDF-1 knock-out efficiency



No efficient knock-out of SDF-1  
(NO GO Decision)



Efficient knock-out of SDF-1  
(GO Decision)

### 2. Knock-out of SDF-1 during experimental glomerulosclerosis



No significant effects regarding  
disease progression  
(NO GO Decision)



Significant effects regarding  
disease progression  
(GO Decision)

### 3. Repeat experiments for receptor (CXCR4/CXCR7) knock-out

### **Key objective 3. Evaluate the role of SDF-1 in tubular cell regeneration (animal procedure 2)**

Tubular cell damage is accompanied by an increased expression of SDF-1. In order to be able to study the relevance of the tubular SDF-1 expression, a conditional nephron-specific SDF-1 knock out (KO) mouse will be generated. This mouse allows inducible nephron-specific SDF-1 knock-down upon doxycycline treatment. For this purpose SDF-1 floxed (SDF-1<sup>fl/fl</sup>) mice will be crossed with the tubule cell promoter mouse. First of all the expression of SDF-1 after doxycycline treatment to knock-out SDF-1 will be analysed and compared to wild type mice. Of note, only if the expression of SDF-1 is reduced significantly within the glomeruli of the kidneys the mice will be suitable for further studies **(GO/NO GO Decision)**.

In these mice, tubular cell injury will be induced using for example the I/R model and/or the glycerol-induced model (depended on the findings of Key objective 1). SDF-1 deficiency will be induced prior to observed changes in the expression of SDF-1 upon disease induction (based on the findings of Key objective 1). Non-transgenic littermates and/or animals that had not received doxycycline will serve as controls. This will allow us to answer if SDF-1 is essential for tubular epithelial cell survival and regeneration in tubular injury. Only if significant effects of SDF-1 knock-out during experimental tubular injury are observed, the same procedures will be performed with CXCR4 and CXCR7 conditional KO mice to determine which receptor pathway is responsible for the observed effects **(GO/NO GO-Decision)**.

To test which receptor pathways mediate the effects of SDF-1 in tubular cell injury and regeneration, CXCR4 or CXCR7 deficiency will be induced. These mice are crossbreds of conditional nephron specific mice and the CXCR4<sup>fl/fl</sup> and/or CXCR7<sup>fl/fl</sup> mice. This will lead to deletion of the CXCR4 and/or CXCR7 gene in our cells of interest. The efficiency of the knock out will be verified by real time PCR and Western blot analysis of isolated proximal tubule cells as well as histological analysis. Also in these mice tubular injury will be induced. Littermates lacking the Cre transgene will serve as controls for the experiment. This will allow us to answer via which receptor (CXCR4 or CXCR7) the observed effects in tubular injury are mediated. The experiments of this key objective are shortly summarized in flow schema 3.

### **Flow schema 3: Schematic illustration of Key objective 3:**

### **Key Objective 3:**

Evaluate the role of the SDF-1/CXCR4/CXCR7 axis in tubular injury



#### **1. Validation of SDF-1 knock-out efficiency**



No efficient knock-out of SDF-1  
(NO GO Decision)



Efficient knock-out of SDF-1  
(GO Decision)

#### **2. Knock-out of SDF-1 during experimental tubular injury**



No significant effects regarding  
disease progression  
(NO GO Decision)



Significant effects regarding  
disease progression  
(GO Decision)

#### **3. Repeat experiments for receptor (CXCR4/CXCR7) knock-out**

**Key objective 4: The effects of SDF-1/CXCR4 and CXCR7 knockout on inflammation and fibrosis after injury and on the microvascular integrity**

Kidney injury (glomerular as well as tubular injury) is accompanied by increased inflammation, progression of kidney fibrosis and goes hand in hand with loss of microvascular integrity. Therefore, all tissue derived from the experiments of key objective 2 and key objective 3 will additionally be scored for inflammation, fibrosis and microvascular integrity to determine the role of SDF-1 in disease progression.

To analyse the effect of SDF-1 knock-down on inflammation and fibrosis of the kidney, biomarker analysis for tubular injury will be performed and the expression of KIM-1 and vimentin will be assessed by immunohistochemistry. It will be scored for the degree of tubular atrophy and interstitial fibrosis. Furthermore, the effects of influx of inflammatory cells will be examined in the early phase as well as the late phase of the injury models. The number of inflammatory cells (PMNs, macrophages and T cells) will be examined by immunohistochemistry with specific markers and by FACS analysis of kidney suspensions.

Additionally, the integrity of the endothelial cells, including their glycocalyx, will be analysed at the ultrastructural level using electron microscopy (EM). The number of proliferating (Ki-67 staining) and apoptotic endothelial cells (TUNEL assay and Caspase-3 staining) will be quantified as well. Furthermore, the number of capillaries in CD31/CD34 double immunostained kidney sections will be determined. The glycocalyx will be analysed using glycocalyx-specific probes and techniques available at the Department of Nephrology. This will allow us to answer if SDF-1 is essential for the integrity of the kidneys microvasculature.

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3.4.3 Describe the coherence between the different components and the different steps of the project. If applicable, describe the milestones and selection points

The four key objectives described in 3.4.2 are complementary in gaining more insight in the role of the SDF-1/CXCR4/CXCR7 axis in the pathophysiological processes of kidney disease. Because of the fact that SDF-1 acts on different cell types (PECs, podocytes, proximal tubular cells,

endothelial cells) and could play a role in different pathophysiological conditions (glomerulosclerosis, microvascular integrity, tubular damage, fibrosis), the analysis of all four subparts is necessary to get a full understanding of the importance of this axis in health and disease. Together, the results will enhance our understanding on the role of the SDF-1 axis in the kidney in health, disease, repair and regeneration. All four key objectives are necessary to address different parts of the pathophysiology in kidney injury and are important to understand the role SDF-1 plays for each cell type and type of injury. Therefore, all topics must be analysed to contribute to a full understanding of the importance of this axis and to answer the general research goal. Also this study is aimed to find possible new pharmaceutical targets to facilitate regeneration and repair and inhibit the progression of the disease which could be tested in a follow-up study.

Importantly, the different sub-projects depend on each other with GO/NO GO-Decisions wherever possible.

**The four key objectives of this project will allow us to approach the research question from different angles. In the end, we expect that the results will synergize with each other and provide us with a broader view on how the SDF-1 axis is involved in maintaining structure and function of renal structures in health and disease.**

3.4.4 List the different types of animal procedures. Use a different appendix 'description animal procedures' for each type of animal procedure.

| Serial number | Type of animal procedure                            |
|---------------|-----------------------------------------------------|
| 1             | SDF-1 signaling in glomerulosclerosis               |
| 2             | SDF-1 signalling in tubular injury and regeneration |

## Appendix

### Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website [www.zbo-ccd.nl](http://www.zbo-ccd.nl).
- Or contact us by phone. (0900-2800028).

## 1 General information

|     |                                                                                                                                  |                                            |                                                                   |
|-----|----------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------|-------------------------------------------------------------------|
| 1.1 | Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.                                     | 10300                                      |                                                                   |
| 1.2 | Provide the name of the licenced establishment.                                                                                  | Stichting Katholieke Universiteit Nijmegen |                                                                   |
| 1.3 | List the different types of animal procedures.<br>Use the serial numbers provided in Section 3.4.4 of the Project Proposal form. | Serial number<br>1                         | Type of animal procedure<br>SDF-1 signaling in glomerulosclerosis |

## 2 Description of animal procedures

### A. Experimental approach and primary outcome parameters

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Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

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The aims of this animal procedure are to determine the expression profile of SDF-1 and its receptors in mouse models of glomerulosclerosis (Aim 1) and to investigate the role of SDF-1 signalling in glomerular function (Aim 2) as well as pathology (glomerulosclerosis) (Aim 3). These aims belong to Key objective 1, 2 and 4.

#### ***Aim 1) The expression profile of SDF-1 and its receptors in glomerular diseases***

Renal diseases are frequently characterized by glomerulosclerosis. One hallmark which goes hand in hand with tubular and glomerular injury is the rarefaction of the renal microvasculature. SDF-1 is a proposed candidate to hinder the scarring of the glomerulus and maintain the microvascular structures. The aim of this procedure is to unravel the role of SDF-1 and its receptors in the kidney in health and disease.

To determine the expression of SDF-1 and CXCR4 as well as CXCR7 in the pathology of glomerulosclerosis, different models of kidney disease will be used to compare the expression profile of SDF-1 and its receptors with the healthy situation. SDF-1 is not easy to detect in tissue using commercially available antibodies, therefore a SDF-1-DSRed reporter mouse will be useful to validate the expression of SDF-1 and enable us to study the cellular source of SDF-1 in health and disease. The targeted mutated DSRed reporter mouse express DSRed from the endogenous SDF-1 promoter, which makes this strain useful in marking cellular sources of SDF-1, as the bright fluorescence can be detected either directly or through anti-RFP (Red-fluorescent protein) immunohistochemistry. Only heterozygous mice will be bred as complete knock-out of SDF-1 during the developmental stage results in a lethal phenotype. The heterozygous mice that we will use for this study have no reported phenotype or signs of discomfort (own observations). CXCR4 and CXCR7 can be stained in mouse tissue using commercially available antibodies. Therefore, the expression of CXCR4 and CXCR7 will be analysed in wild type animals. As the expression of both receptors can be examined in the same tissue, less animals are needed.

To study the expression of SDF-1, CXCR4 and CXCR7 in glomerulosclerosis in mice, we will use different models for scarring of the glomerulus in mice (e.g. the Adriamycin model or Thy1.1. model). Expression of SDF-1 and its receptors will be determined in the kidney tissue at different time points after induction of the disease.

### ***Aim 2) The role of SDF-1 signalling in glomerular function.***

2.1. For this purpose, SDF-1fl/fl mice will be crossed with the podocyte reporter mouse (podocyte-rtTA-LC1 transgenic mouse). In these mice we will be able to stop SDF-1 expression in podocytes when mice are treated with doxycycline. First of all, we will validate the efficiency of the SDF-1 knock-out mouse using immunohistochemistry and methods for RNA and protein detection. Only if the expression of SDF-1 is reduced significantly within the glomeruli of the kidneys the mice will be suitable for further studies **(GO/NO GO Decision)**.

2.2 To study the role of SDF-1 in glomerular capillary maintenance we will treat 6 week old mature mice with doxycycline to knock-out SDF-1 expression within the podocytes. These mice will be monitored for the development of proteinuria and loss of renal function (e.g. serum cystatin C ELISA) and kidneys will be sampled for (immune)histological analysis on (ultra)structural level.

In the mature glomeruli, we will assess in particular the effects of SDF-1 deficiency on the integrity of the glomerular capillaries. The phenotype of the endothelial cells will be examined on ultrastructural level using electron microscopy but also by immunostaining for specific markers normally expressed by endothelial cells i.e. CD31, and PV-1. The latter marker is normally not expressed in the glomerular capillaries but strongly expressed by damaged and immature glomerular endothelial cells in models of glomerular injury (unpublished observation). The data obtained in the conditional KO mice will be compared to mice that are wild type for the target SDF-1 floxed gene.

### ***Aim 3) The role of SDF-1 signalling in glomerulosclerosis.***

3.1. To study the role of SDF-1 in the development of glomerulosclerosis, we will use again the podocyte specific SDF-1 conditional knock-out (KO) mice. In the conditional KO mice glomerulosclerosis will be induced using for example the Adriamycin model. SDF-1 expression will be inactivated shortly before induction of the disease model. Animals that are wild type for the SDF-1 floxed gene will serve as controls for the experiments. At e.g. four different time points (after e.g. 1, 2, 3 and 4 weeks) , the mice will be sacrificed. Those time points were determined in aim 1 and the efficiency of the knock-out was already established in aim 2.1. All animals will be analysed for renal function parameters (proteinuria, albuminuria, serum creatinine levels), and for glomerular injury and glomerulosclerosis as main histological outcome parameters.

3.2. If significant effects on disease progression in the SDF-1 podocyte specific conditional knock-out mice can be detected (in aim 3.1) **(GO/No GO-decision)** we will test which receptor pathways mediate the effects of SDF-1 in glomerulosclerosis. Therefore, mice in which the PECs are deficient for CXCR4 or CXCR7 will be analysed. These mice are crossbreds of the PAX8-Cre mice and the CXCR4fl/fl and/or CXCR7fl/fl mice. This will lead to

deletion of the CXCR4 and/or CXCR7 gene in all epithelial cells of the nephron including the PECs. Furthermore, it is known that CXCR7 is highly expressed on podocytes but the main role of this receptor in the pathogenesis of glomerulosclerosis remains unclear. Therefore, also podocyte specific CXCR7 knockout mice will be created by crossbreeding of the Pod-rtTA mice and the CXCR7<sup>fl/fl</sup> mice. First of all, the efficiency of these knock-out mice will be evaluated by immunohistochemistry as well as methods for RNA and protein detection. Only if the expression of SDF-1, CXCR4 or CXCR7 is reduced significantly within the glomeruli of the kidneys the mice will be suitable for further studies **(GO/NO GO Decision)**.

3.3 In these knock-out mice, glomerulosclerosis will be induced using Adriamycin or the Thy1.1. transgenic mouse model (determined in Aim 1) and the same parameters as described above for the SDF-1 conditional KO mice will be examined.

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Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

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***Experiment 1. Determination of the expression of SDF-1 and its receptors in models of glomerulosclerosis (project proposal, key objective 1).***

To address the difference in pathologies that may lead to glomerulosclerosis we will also use different but well established models of glomerulosclerosis. For this, we will use two different models of glomerulosclerosis, i.e. (a) the adriamycin model that is based on toxic injury to the glomerular epithelial and endothelial cells leading to scarring of the glomerular tuft and (b) the Thy-1.1. transgenic mouse model that is based on primary podocyte injury and subsequent proliferation of parietal epithelial cells. After induction of glomerular injury, we will determine the expression profile of SDF-1 and its receptors in glomerulosclerosis and compare them to the healthy situation. This will enable us to determine the expression level of SDF-1 and its receptors in experimental glomerular disease (aim 1).

***a) Adriamycin model***

The route of administration of Adriamycin is intravenous via the tail vein. One single injection of e.g. 10 mg/kg Adriamycin can be used to induce glomerular injury. Control mice will be treated with a vehicle. Furthermore, baseline blood will be collected via tail cut and mice will be placed in a metabolic cage for max. 24h to collect baseline urine. After this, mice are allowed to recover which can be followed by another urine collection for 24 hours. After the last urine collection, mice will be sacrificed for blood and tissue collection. As described in earlier studies, Adriamycin induces proteinuria and damage of glomerular filtration structures which can be observed as early as 1-2 weeks after Adriamycin injection and are severe after 4 weeks. Therefore, mice will be sacrificed at a maximum of 4 different time points (for example at day 7, 14, 21 and 28 after Adriamycin injection). Tissues will be collected and analysed for the expression of the SDF-1 axis using histological methods and methods for the quantification of RNA and protein levels. The severity of injury will also be determined using specific injury markers such as vimentin or KIM-1.

### ***b) Thy1.1. model***

Another established model to induce glomerulosclerosis is the anti-Thy 1.1 mouse which was successfully used earlier by our group. 5-week-old transgenic mice will receive a single Injection of 1 mg Anti-Thy1.1. antibody in 0.9% saline solution (total volume 0.1 ml). Transgenic mice injected with 0.9% saline solution alone will be used as controls. Furthermore, baseline blood will be collected via tail cut and mice will be placed in a metabolic cage for max. 24h to collect baseline urine. After this, mice are allowed to recover which can be followed by another urine collection for 24 hours. After the last urine collection, mice will be sacrificed for blood and tissue collection. Mice will be sacrificed at a maximum of 4 different time points (for example at day 0, 3, 7 and 21 after injection of the Anti-Thy1.1. antibody). Tissue will be collected and analysed for the expression of the SDF-1 axis using histological methods and methods for the quantification of RNA and protein levels. The severity of injury will also be determined using specific injury markers such as vimentin or KIM-1.

### ***Experiment 2. Characterization of the podocyte-, PEC- and kidney epithelial cell-specific (inducible) knock-out mice for SDF-1 or its receptors (project proposal, key objective 2).***

Before we can study the effect of the podocyte specific SDF-1 knockout on kidney function and injury, we need to validate the knockout of SDF-1 in the kidneys of the mice after doxycycline treatment. For this, 6 week old mice will receive doxycycline. We have several years of experience with doxycycline inducible models and know that supplementation of doxycycline to the drinking water is sufficient to induce recombination in these models. After doxycycline treatment there will be a wash-out phase of seven days. This period is needed to minimize systemic concentrations of doxycycline. Two weeks after the doxycycline treatment the mice will be sacrificed and tissues will be collected. The efficiency of the KO will be analysed by mRNA and protein analysis for SDF-1 on isolated glomeruli from the kidney. This experiment will enable us to evaluate the efficiency of the SDF-1 knock-out mice (aim 2.1). Of note, only if the expression of SDF-1 is reduced significantly within the glomeruli of the kidneys the mice will be suitable for further studies (GO/NO GO Decision).

The same procedures will also be performed with for the mice in which we knock-out the receptors for SDF-1, i.e. CXCR4 and/or CXCR7, respectively. This experiment will enable us to evaluate the efficiency of the CXCR4 and CXCR7 knock-out mice (aim 3.2). Of note, only if the expression of CXCR4 or CXCR7 is reduced significantly within the glomeruli of the kidneys the mice will be suitable for further studies (GO/NO GO Decision).

### ***Experiment 3. The role of SDF-1 signalling in glomerular function in health and disease (project proposal, key objective 2 and 4).***

Six-week old mice will receive doxycycline via drinking water for 7 days followed by a wash out phase of another 7 days. Wild type mice of the flox gene will serve as a control.

**Experiment 3.1** In one group of these doxycycline-treated mice we will study the effect of podocyte specific SDF-1 KO on the normal glomerular function and structural integrity. Therefore, mice will be monitored over time to see if the normal kidney function is affected. At day 0, 7, and 21 and eventually later time points, kidneys will be sampled, and plasma and urine will be collected. If later time points are necessary we will test weekly the urine samples with dipstick urine testing (spontaneous urine flow samples when a mice is handled). Only when dipsticks analysis reveals loss of protein the mice will be placed in a metabolic cage for quantitative assessment of the urine and sacrificed to collect the tissue samples. With this study we will investigate the role of SDF-1 on normal glomerular structure and function (aim 2.2).

**Experiment 3.2** In another group of doxycycline-treated mice, we will monitor the effect of SDF-1 knock-out for the progression and development of glomerulosclerosis. These mice will receive Adriamycin or Thy-1.1 antibody as described above (Experiment 1a and 1b, respectively). Baseline blood and urine will be collected and mice will be sacrificed after the last urine collection for tissue collection. Tissue will be analysed for the expression of the SDF-1 axis using histological methods and methods for the quantification of RNA and protein levels. The severity of injury will also be determined using specific injury markers such as vimentin or KIM-1. With this experiment we will be able to determine the importance of SDF-1 on disease progression and development of glomerulosclerosis (aim 3.1). If significant effects in this model can be observed, the same procedures will be performed with CXCR4 and CXCR7 conditional KO mice (Pod-rtTA or PAX8-CRE mice) to determine which receptor pathway is responsible for the observed effects (GO/NO GO-Decision) (aim 3.3).

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Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

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The number of animals needed to reach statistical significance was determined by performing power calculations. For the power calculations of each experiment we determined the coefficient of variation and effect size using data from literature and previous experiments. For the statistical analysis of the final design of each experiment a biostatistician will be consulted.

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## B. The animals

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Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

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**Species:** SDF-1<sup>tm2.1Sjm/J</sup> mice (SDF-1/DSRed mice) and Pod- or PAX8-rtTA mice as well as the Thy1.1. mice model will be used to determine the expression profile of SDF-1 and its receptors in health and disease. Furthermore, SDF-1<sup>fl/fl</sup> mice will be crossed with the podocyte reporter mouse (podocin-rtTA-LC1 transgenic mouse) or with the PAX8-CRE mice. The same will be done with CXCR4<sup>fl/fl</sup> and CXCR7<sup>fl/fl</sup> mice. Both male and female mice will be used, since we do not expect gender differences in the pathology of the renal disease.

Of note: All mice are back-crossed towards the SV129 background for less variation between mice models and more comparable results in the end.

Note: The estimated number of animals only includes the animals needed to perform experiments, not for breeding. The wildtype mice needed to generate the F1 offspring are not included, since they will only be used for breeding.

We will use a mice model as laboratory animal to study the role of SDF-1 and its receptors in kidney disease, because of its similarity to humans regarding kidney development, organisation in different kidney segments, as well as function, such as filtration of proteins by the glomerulus and subsequent reabsorption by the proximal tubules. We will use wildtype mice and various genetically modified strains. Studying the effect of SDF-1 and its receptors in the complex pathophysiological conditions of glomerulosclerosis is not possible in 'lower' species to be considered as sufficiently comparable to the human situation. Therefore, even though not every pathological aspect in mice appears to be directly translatable to the human situation, it is still the best model available. The various genetically modified organisms are needed to study the specific effects of SDF-1 and its receptors in glomerular pathology.

**Origin:** DSRed/SDF-1 mice were ordered from The Jackson Laboratory. Thy1.1. mice were present at the CDL in Nijmegen. All other mice were derived from Prof. Dr. M.J. Moeller, department of Nephrology and Clinical Immunology, RWTH University Hospital Aachen, Germany. All mice are bred at the Radboudumc Animal facility in Nijmegen or Overasselt.

### **Estimated number:**

To determine the expression profile of SDF-1 and its receptors in two models of glomerulosclerosis (experiment 1), a group of N=6 mice is needed. The final sample size per group should be adjusted for expected attrition, resulting in N=7 mice per group. The experiment will be performed in DSRed mice and SV129 mice for the Adriamycin model and in the Thy1.1. mice. Analysis of the expression will be performed at a maximum of 4 different time points, resulting in an amount of N=28 mice per model which gives a total amount of N=84 mice for experiment 1.

To test the efficiency of the 5 different (inducible) knock-out lines and appropriate controls, we need a final sample size of N=35 mice for experiment 2.

If the results of experiment 2 are sufficient to continue with experiment 3 (minimal 90% knock-out observed in the glomerulus of SDF-1 or the responsible receptors compared to wild type mice), we will establish in Experiment 3.1 the effects of podocyte specific SDF-1 KO on the normal function and integrity of the glomerulus. The structural integrity and glomerular function will be assessed at i.e. day 0, 7 and 21. The effects will be compared with control mice wt for the SDF-1 floxed gene, resulting in 6 different groups. The groups will be N=8 after correcting for expected attrition. This gives a final sample size of N=48 for experiment 3.1.

In experiment 3.2 we will determine the effects of SDF-1, CXCR4 or CXCR7 knockout in a model of experimental glomerulosclerosis. The development of glomerulosclerosis is induced by Thy1.1. and/or Adriamycin. The Adriamycin model is associated with some variation mainly due the narrow therapeutic index of Adriamycin and also in the Thy1.1. model some variation in disease severity can be expected. Based on the experience in previous experiments we have set the variation coefficient for the models on 15%. The effect size is difficult to determine due to lack of information but however should be 20% or more to be relevant. Power calculation results in N=13 for each group. The final sample size per group should be adjusted for expected attrition which gives a final sample size of N=15 mice per group. We will use maximal four different time points as well as knock-out mice for SDF-1, CXCR4, CXCR7 and a combination of both receptors and compare the pathologic situation with the healthy situation. This gives a maximum final sample size of N=420 mice for experiment 3.2.

In summary, the maximum number of animals which will be used is N=587 mice. Depending on the GO/NO GO Decisions, lower numbers may be expected.

Life stages: Our experiments will preferably be performed in mice younger than 3 months. Mice will not be allowed to grow older than one year. Both female and male mice will be used.

| Species                 | Origin                                                                                                                      | Maximum number of animals | Life stage |
|-------------------------|-----------------------------------------------------------------------------------------------------------------------------|---------------------------|------------|
| Pod-rtTA/LC1-SDF-1fl/fl | Prof. Dr. M. J. Moeller, Department of Nephrology and Clinical Immunology, RWTH University Hospital Aachen, Aachen, Germany | 89                        | 6-10 weeks |
| Pod-rtTA/LC1-CXCR7fl/fl | Prof. Dr. M. J. Moeller, Department of Nephrology and Clinical Immunology, RWTH University Hospital Aachen, Aachen, Germany | 65                        | 6-10 weeks |

|                                     |                                                                                                                             |    |            |
|-------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|----|------------|
| PAX8-rtTA/CRE-CXCR4fl/fl            | Prof. Dr. M. J. Moeller, Department of Nephrology and Clinical Immunology, RWTH University Hospital Aachen, Aachen, Germany | 65 | 6-10 weeks |
| PAX8-rtTA/CRE-CXCR7fl/fl            | Prof. Dr. M. J. Moeller, Department of Nephrology and Clinical Immunology, RWTH University Hospital Aachen, Aachen, Germany | 65 | 6-10 weeks |
| PAX8-rtTA/CRE-CXCR4fl/fl-CXCR7fl/fl | Prof. Dr. M. J. Moeller, Department of Nephrology and Clinical Immunology, RWTH University Hospital Aachen, Aachen, Germany | 65 | 6-10 weeks |
| SDF-1/DSRed                         | The Jackson Laboratory                                                                                                      | 28 | 6-10 weeks |
| Thy1.1.                             | CDL Nijmegen                                                                                                                | 28 | 5-8 weeks  |
| SV129                               | Charles River                                                                                                               | 28 | 6-10 weeks |
| PAX8-rtTA/CRE                       | Prof. Dr. M. J. Moeller, Department of Nephrology and Clinical Immunology, RWTH University Hospital Aachen, Aachen, Germany | 65 | 6-10 weeks |
| Pod-rtTA/LC1                        | Prof. Dr. M. J. Moeller, Department of Nephrology and Clinical Immunology, RWTH University Hospital Aachen, Aachen, Germany | 89 | 6-10 weeks |

### C. Re-use

Will the animals be re-used?

☒ No, continue with question D.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

☐ No

☐ Yes > Provide specific justifications for the re-use of these animals during the procedures.

## **D. Replacement, reduction, refinement**

---

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

---

### ***Replacement***

A significant part of our research is performed with in vitro cultures using cell-lines. To evaluate the role of SDF-1, we will functionally test the effect of SDF-1 stimulation as well as inhibition on cultured podocytes and parietal epithelial cells under physiological and pathophysiological (toxic and hypoxic) conditions. Different assays to determine cell viability, metabolic activity, cell proliferation and migration will be used. This will provide us with information which can be used for the preparation of the animal experiments. Furthermore, the effects seen in vitro can be compared with the in vivo data. Therefore, we will first test the effects of pathway stimulation and inhibition in vitro to avoid unnecessary animal experiments and to determine which cell type is most susceptible to SDF-1 pathway inhibition/stimulation. However, we still need a whole organism to be able to study and differentiate between the effects of SDF-1 and its receptors on kidney injury. Studying the effect of SDF-1 and its receptors in the complex pathophysiological conditions of glomerulosclerosis is not possible in 'lower' species than mice to be considered as sufficiently comparable to the human situation. Therefore, even though not every pathological aspect in mice appears to be directly translatable to the human situation, it is still the best model available. The various genetically modified organisms are needed to study the cell specific effects of SDF-1 and its receptors in kidney pathology. Also the consequences of inhibition of this axis on kidney function cannot be mimicked in vitro. The mouse is known to resemble renal injury observed in humans and has an adequate size to study the kidney extensively.

### ***Reduction***

Prior to each experiment we will perform a literature search to find all information relevant for the animal experiment. In addition, a power calculation will be performed based on data from literature and previous experiments to determine the minimum amount of animals needed. Furthermore, phenotype analysis in mice is only applied when genotype analysis confirms mutations in the targeted gene. Also, we strive to make optimal use of the material from each mouse by combining experiments wherever possible. One example is the high amount of different histological parameters which will be analysed after each experiment such as fibrosis, microvasculature and inflammation (see project proposal, outline, key objective 4). This will provide us with information useful to get a broader overview of the pathophysiological role of the SDF-1 pathway without the need to perform new animal experiments. Furthermore, to use tissues optimally we will test our methods and antibodies prior to all experiments on tissues collected in previous experiments. These tissues have been collected and archived enabling us to test protocols and reagents for the assays that will be performed on the newly collected tissues within the proposed experiments. Also, we will minimize the number of animals needed by first choosing the best available animal model to study the expression of the SDF-1 pathway in health and disease (experiment 1), second, performing validation of the knockout of SDF-1 in the kidneys of the mice after doxycycline treatment (experiment 2), and third, studying the effect of SDF-1

and its receptors in glomerular function also during pathologic condition (experiments 3.1 and 3.2). These three steps are not performed parallel but will be performed step-by-step with a GO/No GO decision after each part.

### ***Refinement***

Most importantly to mention is, that we have a cell specific inducible knock-out mice which allows representative cell-specific and disease specific results in a time-dependent manner. Also, this prevents developmental malformations in the kidneys and we will for the first time be able to study this pathway in adult mice because of the fact that a complete knock-out during development of SDF-1 and its receptors results in a lethal phenotype. To reduce anxiety, mice will be housed in groups and not individually. The general health of the animals will be monitored daily by the researcher and/or the biotechnician. The methods that we use are well established and the experiments will be performed by experienced researchers. Previous research by our group and others has shown the translational value of this type of studies. For the collection of urine, mice need to be placed in a metabolic cage for 24 hours . Shelters will be placed in the metabolic cages to reduce distress and enhance animal welfare.

---

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

---

In order to prevent unnecessary discomfort or stress, the general health of the animals will be checked daily by the researchers or the biotechnicians. In case animals reach a humane end point (described in J) the responsible researcher will be contacted. To reduce anxiety, mice will be housed in groups and not individually.

Despite the formation of renal injury, no suffering or pain is expected within the time frame of the study, based on previous work and the fact that patients with similar pathology are also not treated with pain relieving medication. Fear could be expected from placement in the metabolic cage. This will be minimized by placing shelters in the metabolic cage. Furthermore, in experiments where mice are monitored over a longer time we will test the urine by dipstick analysis on spot urine (spontaneous flow) instead of placement of the mice in a metabolic cage. Only when proteinuria is detected, mice will be placed in a metabolic cages. No adverse effects on the environment are expected.

## **Repetition and Duplication**

## E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

The experiments described in this application follow a sequence and do not run in parallel with important GO-No GO decisions wherever possible. The experiments will be well documented and tissues will be systematically stored in a tissue bank. In this way we and others can use the collected tissues of the injury models in other studies preventing the need for another animal experiment. Furthermore, based on literature study, we know that these experiments have not been performed earlier in other studies.

## Accommodation and care

### F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

☒ No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

### G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

☒ No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

## Classification of discomfort/humane endpoints

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**H. Pain and pain relief**

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Will the animals experience pain during or after the procedures?

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☐ No > Continue with question I.

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

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☒ No > Justify why pain relieving methods will not be used.

---

Minor pain may be expected from the injections, no pain relief will be applied for this.

☐ Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

---

## I. Other aspects compromising the welfare of the animals

---

Describe which other adverse effects on the animals welfare may be expected?

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Injection pain

Blood collection

Metabolic cage

Kidney disease

Gene knock-down (No effects on welfare are expected due to the cell specific and inducible nature of the knock-out mouse models used in this proposal)

Explain why these effects may emerge.

---

Injection pain: Due to injection of Adriamycin or Thy1.1. antibody, mice may experience an injection pain. Rarely, injection via the tail vein can result in tail necrosis. This is a very rare event, but it has to be mentioned here as one possibility. As we have good experienced and trained biotechnicians who will carry out this procedure, development of a tail necrosis after iv injection is very unlikely.

Blood collection: The blood will be collected via tail cut. Mice may experience discomfort during this procedure.

Metabolic cage: The mice may experience discomfort (i.e. fear) due to being placed in a metabolic cage. To reduce discomfort in the metabolic cage, a shelter will be provided.

Kidney disease: Although the mice will develop glomerulosclerosis, this is unlikely to compromise their welfare within the time frame of this study, based on previous work and data of patients with the same pathology.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

---

The animal procedures will only be performed by experienced researchers or biotechnicians (i.e. injection or blood collection). Researchers will be trained prior to perform the animal procedures by experienced biotechnicians. Furthermore, social housing will be performed wherever possible to prevent the occurrence of stress and discomfort. Although mice could experience discomfort or stress by being placed in individual metabolic cages. To reduce this, shelters will be placed on the metabolic cages.

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**J. Humane endpoints**

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May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

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☐ No > Continue with question K.

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☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

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The general humane endpoints apply:

1. The animal experiences discomfort as a result of conditions not related to the experiment (e.g. injuries/wounds/infections)
2. The animal experiences more discomfort than justified for the purpose of the experiment and weighed by the DEC.
3. (reliable and applicable) results cannot be achieved because of conditions not related with the experiment

Experiment-related humane endpoints apply:

1. Kidney failure or severe decrease of kidney function due to induction of glomerulosclerosis. This endpoint will apply if the mice become edemic, behave ill, are not motile and/or are sitting apathetic in the cage.

---

Indicate the likely incidence.

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The induction of glomerulosclerosis can lead to a reduced kidney function. In this case mice will become edemic. In regard to the disease models mentioned in this proposal we have observed this only in a few cases (less than 5%). Furthermore, a knock-out phenotype could rarely lead to specific unexpected defects which may indicate a humane endpoint but this is very unlikely as it was until now not observed in these mice.

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#### **K. Classification of severity of procedures**

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Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned (non-recovery, mild, moderate, severe ).

---

Based on the individual procedures listed below, we anticipate a cumulative level of discomfort that is mild for all mice. 0% of the animals will develop a moderate or severe level of discomfort.

- Tail vein injection: mild
- Housing in metabolic cage: mild
- Blood collection (baseline): mild
- Doxycycline ip.: mild
- Sacrificing: mild

| Experiment          | Exp. 1    |           |            | Exp. 2    |           | Exp. 3.1  |           | Exp. 3.2    |                   |
|---------------------|-----------|-----------|------------|-----------|-----------|-----------|-----------|-------------|-------------------|
| Group               | Ctr       | Thy1.1.   | Adriamycin | Ctr       | Knock-out | Ctr       | Knock-out | Disease-ctr | Disease-knock-out |
| Nr. animals         | <b>14</b> | <b>28</b> | <b>42</b>  | <b>10</b> | <b>25</b> | <b>24</b> | <b>24</b> | <b>120</b>  | <b>300</b>        |
| Tail vein injection | Yes       | Yes       | Yes        | No        | No        | No        | No        | Yes         | Yes               |
| Metabolic cage      | Yes       | Yes       | Yes        | No        | No        | Yes       | Yes       | Yes         | Yes               |
| Blood collection    | Yes       | Yes       | Yes        | No        | No        | Yes       | Yes       | Yes         | Yes               |

## End of experiment

### L. Method of killing

Will the animals be killed during or after the procedures?

☐ No > Continue with Section 3: 'Signatures'.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

It is necessary to kill the animals at the end of the experiment to collect tissue needed for analysis and for data collection. Importantly, we need the kidneys of the mice and also we have to collect blood from the mice. Therefore, to avoid suffering from more animal procedures it is necessary to kill the animals at the end of the experiment.

☐ Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☒ Yes

## Appendix

### Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website [www.zbo-ccd.nl](http://www.zbo-ccd.nl).
- Or contact us by phone. (0900-2800028).

# 1 General information

|     |                                                                                                                                  |                                            |                                                                                 |
|-----|----------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------|---------------------------------------------------------------------------------|
| 1.1 | Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.                                     | 10300                                      |                                                                                 |
| 1.2 | Provide the name of the licenced establishment.                                                                                  | Stichting Katholieke Universiteit Nijmegen |                                                                                 |
| 1.3 | List the different types of animal procedures.<br>Use the serial numbers provided in Section 3.4.4 of the Project Proposal form. | Serial number<br>2                         | Type of animal procedure<br>SDF-1 signalling in tubular injury and regeneration |

## 2 Description of animal procedures

### A. Experimental approach and primary outcome parameters

---

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

---

The aims of this procedure are to determine the expression profile of SDF-1 and its receptors in mouse models of tubular injury (aim 1) and to investigate the role of SDF-1 signalling in tubular disease (aim 2). These aims belong to Key objectives 1,3 and 4 described in the project proposal.

#### ***Aim 1) The expression profile of SDF-1 and its receptors in experimental acute tubular injury***

First of all, we will analyse the expression of SDF-1, CXCR4 and CXCR7 in models for acute kidney injury (AKI): e.g. ischemia reperfusion (I/R) model and glycerol-induced AKI model. The I/R model is a model of hypoxic tubular injury, caused by kidney hypoperfusion normally observed in ischemia and/or kidney transplantation and can be induced by clamping the renal blood supply for approximately 30-40 minutes followed by reperfusion after release of the clamp. The glycerol model is a model of toxic tubular injury, caused by myoglobin, as seen in rhabdomyolysis. The glycerol model is induced by injection of glycerol in the hind legs of the mice inducing rhabdomyolysis and subsequent proximal tubular cell injury. Previously we have successfully used both models. Both models will be performed in SDF-1-DsRed reporter mice and also wildtype mice as outlined in experiment 1, which will allow sensitive analysis of expression of SDF-1 and its receptors in time and in location. As SDF-1 is not easy to detect in tissue using commercially available antibodies, a SDF-1-DsRed reporter mouse will be used to validate the expression of SDF-1 and enable us to study the cellular source of SDF-1 in health and disease. Only heterozygous mice will be bred as complete knock-out of SDF-1 during development results in a lethal phenotype. These heterozygous mice have no reported phenotype or signs of discomfort (own and reported observations). CXCR4 and CXCR7 can easily be stained in mouse tissue using commercially available antibodies. Therefore, the experiments which are performed for detection of the expression of CXCR4 and CXCR7 are performed in wild type animals, which also reduces the amount of animals needed as the expression of both receptors can be detected in one tissue.

In addition to histological analysis of the kidney, the expression of SDF-1, CXCR4 and CXCR7 will be assessed at mRNA level (real-time PCR) and at protein level (immunohistochemistry and Western blot). For both models mice will be sacrificed at different time points (e.g. at day 1, 5, 7 and 14) after induction of the injury, and kidneys, plasma and urine will be collected. Based on the findings of these studies, we will select one of the two AKI models (I/R- or glycerol-induced) to use in the following aims. We will choose the model that induces the largest differences in SDF-1, CXCR4 and CXCR7 expression compared to the healthy controls.

***Aim 2) The role of SDF-1 signalling in tubular injury.***

2.1. First of all, we will validate the efficiency of the SDF-1 knock-out mouse using immunohistochemistry and methods for RNA and protein detection. Only if the expression of SDF-1 is reduced significantly within the glomeruli of the kidneys the mice will be suitable for further studies **(GO/NO GO Decision)**.

2.2. To study the role of SDF-1 in the development of tubular injury, we will use a specific SDF-1 conditional knock-out (KO) mice. In the conditional KO mice tubular injury will be induced by either using I/R model or glycerol model. SDF-1 expression will be inactivated shortly before induction of the disease model. Animals that are wild type for the SDF-1 floxed gene will serve as controls for the experiments. At different time points, the mice will be sacrificed. All animals will be analysed for renal function parameters (proteinuria, albuminuria, serum creatinine levels), and for histological tubular injury as well as endothelial cell function and capillary rarefaction as main outcome parameters. In addition, activation and proliferation of tubular cells and endothelial cell phenotype and proliferation will be examined in the KO mice.

2.3. If significant effects in the tubular specific conditional knock-out are detected, **(GO/No GO decision)**, we will test which receptor pathways mediate the effects of SDF-1 in the development of tubular injury. Therefore, mice in which the tubular cells are deficient for either CXCR4 or CXCR7 will be analysed. These mice are crossbreds of the PAX8-Cre mice and the CXCR4<sup>fl/fl</sup> or CXCR7<sup>fl/fl</sup> mice respectively. This will lead to deletion of the CXCR4 or CXCR7 gene in all epithelial cells of the nephron including proximal tubular epithelial cells. First of all, we will validate the efficiency of the SDF-1 knock-out mouse using immunohistochemistry and methods for RNA and protein detection. Only if the expression of SDF-1 is reduced significantly within the tubular epithelial cells of the kidney, the mice will be suitable for further studies **(GO/NO GO Decision)**.

2.4 In these CXCR4 and CXCR7 conditional knock-out mice, tubular injury will be induced using I/R model or glycerol model and the same parameters as described above for the SDF-1 conditional KO mice will be examined (see Aim 2.2).

---

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

***Experiment 1. Determination of the expression of SDF-1 and its receptors in models of tubular injury (project proposal, key objective 1).***

One main complication which is frequently present in patients with acute or chronic kidney injury is the presence of acute tubular injury. There are different models available to induce tubular injury in mice. We will test the glycerol model and the I/R model to induce tubular injury and we will determine the expression profile of SDF-1 and its receptors in the different models and compare them to the healthy situation and to the expression

of this pathway observed in human biopsies of patients with AKI. To validate the expression of SDF-1 and make sensitive analysis of the expression levels observed in experimental renal disease, we will use SDF-1-DSRed reporter mice. To study the expression of the CXCR4 and CXCR7 receptor, no reporter mice will be used.

#### ***a) Glycerol model***

For the glycerol model, mice will be treated with one intramuscular injection of glycerol (7,5 mg/kg 50% glycerol) into the hind limbs. The glycerol model is a model of toxic tubular injury, caused by myoglobin, as seen in rhabdomyolysis. The glycerol model is induced by injection of glycerol in the hind legs of the mice inducing rhabdomyolysis and subsequent proximal tubular cell injury. As already described in Animal procedure 1 for the Adriamycin model, baseline blood will be collected via tail cut and mice will be placed in a metabolic cage for max. 24h to collect baseline urine. After this, mice are allowed to recover for at least 2 days, before induction of the glycerol model. During the experiment, urine is collected one day before the mice will be sacrificed. At the end of the experiment, mice will be sacrificed for blood and tissue collection. It is known that glycerol injection results in acute direct kidney injury with a maximum elevation of BUN and serum creatinine levels after 24 hours which remains elevated for 5 days and normalizes thereafter. Because of this, mice will be sacrificed at a maximum of 5 different time points ( e.g. at day 1, 3, 5, 7 and 14) to look at the expression of SDF-1 and its receptors in a time- and disease-stage-dependent manner.

#### ***b) I/R model***

For the I/R model, unilateral kidney ischemia reperfusion will be induced by clamping left renal pedicles for 30-40 minutes under anaesthesia (e.g. 2% isoflurane). This is followed by reperfusion of the kidney. Mice will be sacrificed at a maximum of 5 different time points (for example day 1, 3, 5, 7 and 14) after the I/R procedure and tissue samples will be collected and analysed.

This experiment will enable us to evaluate the expression of SDF-1 and CXCR4, as well as CXCR7 in the healthy kidney and during experimental renal disease (aim 1).

### ***Experiment 2. Characterization of the PAX8-SDF-1-flox mouse (project proposal, key objective 3).***

2.1 Before we can study the effect of the specific SDF-1 knockout on kidney function and injury, we need to validate the knockout of SDF-1 in the kidneys of the mice after doxycycline treatment. For this, 6 week old mice will receive doxycycline. We have several years of experience with doxycycline inducible models and know that supplementation of doxycycline to the drinking water is sufficient to induce recombination in these models. After doxycycline treatment there will be a wash-out phase of seven days. This period is needed to minimize systemic concentrations of

doxycycline. Two weeks after the doxycycline treatment the mice will be sacrificed and tissues will be collected. The efficiency of the KO will be analysed by immunostaining for SDF-1 on kidney sections and mRNA as well as protein analysis on isolated tubular cells from the kidney.

This experiment will enable us to evaluate the efficiency of the SDF-1 knock-out mice (aim 2.1). Of note, only if the expression of SDF-1 is reduced significantly within the tubuli of the kidneys the mice will be suitable for further studies **(GO/NO GO Decision)**.

2.2 The same procedures will also be performed with CXCR4 and CXCR7 flox mice. This experiment will enable us to evaluate the efficiency of the CXCR4 and CXCR7 knock-out mice (aim 2.3). Of note, only if the expression of CXCR4 and CXCR7 is reduced with at least 90% within the proximal tubular cells, the mice will be suitable for further studies **(GO/NO GO Decision)**.

***Experiment 3. The role of SDF-1 signalling in tubular pathology, regeneration and microvascular integrity (project proposal, key objective 3 and 4).***

If the conditional KO mouse is suitable for the proposed studies, we will treat 6 week old mice with doxycycline. These mice will receive doxycycline via drinking water for 7 days followed by a wash out phase of another 7 days. Wild type mice of the SDF-1 flox gene will serve as a control.

After this, disease induction will be performed for either the I/R model or the glycerol model. Tissue will be collected and analysed for the expression of the SDF-1 axis using histological methods and methods for the quantification of RNA and protein levels. The severity of injury will also be determined using specific injury markers such as vimentin or KIM-1.

Furthermore, to examine the integrity of the endothelial cells, including their glycocalyx, analysis will be performed at the ultrastructural level using electron microscopy (EM). The number of proliferating (Ki-67 staining) and apoptotic endothelial cells (TUNEL assay and Caspase-3 staining) will be quantified as well. In addition, the number of peritubular capillaries in CD31/CD34 double-immunostained kidney sections will be determined. This experiment will help us to evaluate the role of SDF-1 during proximal tubular injury and regeneration (aim 2.2). If significant effects in this model have been observed, the same procedures will be performed with CXCR4 and CXCR7 conditional KO mice **(GO/NO GO Decision)**. This will help us to determine which receptor pathway is responsible for the observed effects (aim 2.4)

---

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

The minimal number of animals needed to reach statistical significance within the experiments was determined by power calculations for each experiment. The variation coefficient and effect size were based on data from literature and from previous experiments. For the final design of the experiments a biostatistician will be consulted.

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## B. The animals

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Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

---

**Species:** SDF-1tm2.1Sjm/J mice (SDF-1/DSRed) and PAX8-rtTA mice will be used to determine the expression profile of SDF-1 and its receptors in health and disease. Furthermore, SDF-1fl/fl mice will be crossed with the PAX8-CRE mice. Also crossbreds of the PAX8-Cre mice and the CXCR4fl/fl or CXCR7fl/fl mice respectively will be used.

Both male and female mice will be used, since we do not expect gender differences in the pathology of the renal disease.

Note: the estimated number of animals only includes the animals needed to perform experiments, not for breeding. The wildtype mice needed to generate the F1 offspring are not included, since they will only be used for breeding.

We will use a mouse model as laboratory animal to study the role of SDF-1 and its receptors in kidney disease, because of its similarity to humans regarding kidney development, cellular organisation in different kidney segments, as well as function, such as filtration of proteins by the glomerulus and subsequent reabsorption by the proximal tubules. We will use wildtype mice and various genetically modified strains. Studying the effect of SDF-1 and its receptors in the complex pathophysiological conditions of glomerulosclerosis is not possible in 'lower' species to be considered as sufficiently comparable to the human situation. Therefore, even though not every pathological aspect in mice appears to be directly translatable to the human situation, it is still the best model available. The various genetically modified organisms are needed to study the specific effects of SDF-1 and its receptors in tubular injury and regeneration.

**Origin:** SDF-1/DSRed mice were ordered from The Jackson Laboratory. All other mice were derived from Prof. Dr. M.J. Moeller, Department of Nephrology and Clinical Immunology, RWTH University Hospital Aachen, Germany. All mice are bred at the Radboudumc Animal facility in Nijmegen and Overasselt.

**Estimated number:**

To determine the expression profile of SDF-1 and its receptors in tubular disease we will induce two different models of tubular injury (experiment 1) in SDF-1-DSRed mice and in wild-type mice. The group size was calculated and we will use N=6 mice. The final sample size per group should be adjusted for expected attrition, resulting in N=7 mice per group for a maximum of 5 time points. This experiment will be performed for the glycerol model, for which we need also a control group of N=7 mice and the I/R model in which no control group is needed, as reperfusion injury is induced unilaterally. This results in a total amount of N=154 mice for experiment 1.

To test the efficiency of the inducible knock-out as mentioned in experiment 2, a group of N=4 for the different knock-out mice (KO for SDF-1, CXCR4 and/or CXCR7) as well as the wild type is needed for comparison. The final sample size per group should be adjusted for expected attrition, resulting in N=5 mice per group for a maximum of 5 groups, which gives a final sample size of N=25 mice for experiment 2.

In experiment 3, we will establish the effects of the SDF-1, CXCR4 or CXCR7 knockout in a model of experimental tubular injury. Power calculation of this comparison resulted in a N=14 for each group. The final sample size per group should be adjusted for expected attrition which gives a final sample size of N=16 mice per group. We will use a maximum of four different time points as well as knock-out mice for SDF-1, CXCR4, CXCR7 and a combination of both receptors and compare the pathologic situation with the pathologic knock-out situation. This gives a final sample size of N=320.

In summary, the maximum number of animals which will be used is N=499 mice. Depending on the GO/NO GO Decision, lower numbers may be expected.

Life stages: Our experiments will preferably be performed in mice younger than 3 months. Mice will not be allowed to grow older than one year.

| Species                  | Origin                                                                                                                      | Maximum number of animals | Life stage |
|--------------------------|-----------------------------------------------------------------------------------------------------------------------------|---------------------------|------------|
| SDF-1-DSRed              | The Jackson Laboratory                                                                                                      | 77                        | 6-10 weeks |
| PAX8-rtTA/CRE-SDF-1fl/fl | Prof. Dr. M. J. Moeller, Department of Nephrology and Clinical Immunology, RWTH University Hospital Aachen, Aachen, Germany | 69                        | 6-10 weeks |
| PAX8-rtTA-CRE-CXCR4fl/fl | Prof. Dr. M. J. Moeller, Department of Nephrology and Clinical Immunology, RWTH University Hospital Aachen, Aachen, Germany | 69                        | 6-10 weeks |
| PAX8-rtTA/CRE-CXCR7fl/fl | Prof. Dr. M. J. Moeller, Department of Nephrology and Clinical Immunology, RWTH University Hospital Aachen, Aachen, Germany | 69                        | 6-10 weeks |

|                                         |                                                                                                                                      |    |            |
|-----------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------|----|------------|
| PAX8-rtTA/CRE-CXCR4fl/fl-<br>CXCR7fl/fl | Prof. Dr. M. J. Moeller, Department<br>of Nephrology and Clinical<br>Immunology, RWTH University<br>Hospital Aachen, Aachen, Germany | 69 | 6-10 weeks |
| PAX8-rtTA/CRE                           | Prof. Dr. M. J. Moeller, Department<br>of Nephrology and Clinical<br>Immunology, RWTH University<br>Hospital Aachen, Aachen, Germany | 69 | 6-10 weeks |
| SV129                                   | Charles River                                                                                                                        | 77 | 6-10 weeks |

### C. Re-use

Will the animals be re-used?

☒ No, continue with question D.

☐ Yes > Explain why re-use is considered acceptable for this animal procedure.

Are the previous or proposed animal procedures classified as 'severe'?

☐ No

☐ Yes > Provide specific justifications for the re-use of these animals during the procedures.

### D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

### **Replacement**

A significant part of our research is performed with in vitro cultures using cell-lines. To evaluate the role of SDF-1, we will functionally test the effect of SDF-1 stimulation as well as inhibition on cultured proximal tubule cells under physiological and pathophysiological (toxic and hypoxic) conditions. Different assays to determine cell viability, metabolic activity, cell proliferation and migration will be used. This will provide us with information which

can be used for the preparation of the animal experiments. Furthermore, the effects seen in vitro can be compared with the in vivo data. Therefore, we will first test the effects of pathway stimulation and inhibition in vitro to avoid unnecessary animal experiments and to determine which effect on cell injury or regeneration can be observed after SDF-1 pathway inhibition/stimulation. However, we need a whole organism to be able to study and differentiate between the effects of SDF-1 and its receptors on kidney injury. Also the consequences of inhibition of this axis cannot be mimicked in vitro. The mouse is known to resemble renal injury observed in humans and has an adequate size to study the kidney extensively.

### ***Reduction***

Prior to each experiment we will perform a literature search to find all information relevant for the animal experiment. In addition, a power calculation will be performed based on data from literature and previous experiments to determine the minimum amount of animals needed. Furthermore, phenotype analysis in mice is only applied when genotype analysis confirms mutations in the targeted gene. Also, we strive to make optimal use of the material from each mouse by combining experiments wherever possible.

We will try to minimize the number of animals needed by first choosing the best available animal model to study the expression of the SDF-1 pathway in health and disease (experiment 1), second, performing validation of the knockout of SDF-1 in the kidneys of the mice after doxycycline treatment (experiment 2), and third, studying the effect of SDF-1 in tubular injury (experiment 3). These four steps are not performed parallel but will be performed step-by-step with a GO/No GO decision after each part.

For the I/R reperfusion model we have chosen to perform the ischemia unilaterally. In this way we have an affected and an control kidney from the same animal. By performing I/R unilaterally we do not need Sham operated control mice.

### ***Refinement***

Most importantly to mention is, that we will use cell specific inducible knock-out mice. This will allow representative cell-specific and disease specific results in a time-dependent manner. Also, this prevents developmental malformations in the kidneys and we will for the first time be able to study this pathway in adult mice because of the fact that a complete knock-out during development of SDF-1 and its receptors results in a lethal phenotype.

To reduce anxiety, mice will be housed in groups and not individually. The general health of the animals will be monitored daily by the researcher and/or the biotechnician. The methods that we use are well established and the experiments will be performed by experienced researchers. Previous research by our group and others has shown the translational value of this type of studies. For the collection of urine, mice need to be placed in a metabolic cage. Shelters will be placed in the metabolic cages to reduce distress and enhance animal welfare.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

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In order to prevent unnecessary discomfort or stress the general health of the animals will be checked daily by the researchers or the biotechnicians. In case animals reach a humane end point (described in J) the responsible researcher will be contacted. To reduce anxiety, mice will be housed in groups and not individually. If necessary, anaesthesia and analgesics will be applied to reduce discomfort.

Despite the formation of renal injury, no suffering or pain is expected within the time frame of the study, based on previous work and the fact that patients with similar pathology are also not treated with pain relieving medication. Pain could be expected from the animal procedures (e.g. operation of the mice for I/R or injection of glycerol) which could make it necessary to treat animals with pain relieving medication. This will be decided individually. Fear could be expected from placement in the metabolic cage. This will be minimized by placing shelters in the metabolic cage. No adverse effects on the environment are expected.

## **Repetition and Duplication**

### **E. Repetition**

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Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

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The experiments described in this application follow a sequence and do not run in parallel with important GO-No GO decisions wherever possible. The experiments will be well documented and tissues will be systematically stored in a tissue bank. In this way we and others can use the collected tissues of the injury models in other studies preventing the need for another animal experiment. Furthermore, based on literature study, we know that these experiments have not been performed earlier by other groups.

## **Accommodation and care**

#### **F. Accommodation and care**

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

☒ No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

#### **G. Location where the animals procedures are performed**

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

☒ No > Continue with question H.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

## **Classification of discomfort/humane endpoints**

#### **H. Pain and pain relief**

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

☒ Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

Minor pain may be expected from the injections, no pain relief will be applied for this. After surgical procedures such as the I/R-model in which unilateral kidney ischemia reperfusion is performed, analgesia will be used to reduce discomfort and pain.

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**I. Other aspects compromising the welfare of the animals**

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Describe which other adverse effects on the animals welfare may be expected?

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Necrosis because of injection of glycerol into the hind limbs (Glycerol model), injection pain, metabolic cage, blood collection

Anxiety, stress and discomfort due to anaesthesia

Surgical procedure / Wound healing (I/R model)

Although the mice will develop tubular injury, this is unlikely to compromise their welfare within the time frame of this study, based on previous work and data of patients with the same pathology.

Explain why these effects may emerge.

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The mice may experience discomfort (i.e. fear) due to being placed in a metabolic cage. To reduce discomfort in the metabolic cage, a shelter will be provided.

Necrosis can occur because of injection of glycerol into the hind limbs (Glycerol model)

Blood collection: The blood will be collected via tail cut. Mice may experience discomfort during this procedure.

Wound healing can lead to some discomfort for the mice as it can occur after surgical procedures.

Although the mice will develop tubular injury, this is unlikely to compromise their welfare within the time frame of this study, based on previous work and data of patients with the same pathology.

Gene knock-down (No effects on welfare are expected due to the cell specific and inducible nature of the knock-out mouse models used in this proposal)

Indicate which measures will be adopted to prevent occurrence or minimise severity.

The animal procedures will only be performed by experienced researchers or biotechnicians (i.e. injection or blood collection, surgical procedures). Researchers will be trained prior to perform the animal procedures by experienced biotechnicians. Furthermore, social housing will be performed wherever possible to prevent the occurrence of stress and discomfort. Although mice could experience discomfort or stress by being placed in individual metabolic cages. To reduce this, shelters will be placed in the metabolic cages. Analgesics will be given to mice after surgical procedure to reduce discomfort from wound healing.

#### **J. Humane endpoints**

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question K.

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

The general humane endpoints apply:

1. The animal experiences more than little, additional, discomfort as a result of conditions not related to the experiment (e.g. injuries/wounds/infections)
2. The animal experiences more discomfort than justified for the purpose of the experiment and weighted by the DEC
3. (reliable and applicable) results cannot be achieved because of conditions not related with the experiment

Experiment-related humane endpoints apply:

1. Wound infection after unilateral kidney ischemia reperfusion.
2. Kidney failure or severe decrease of kidney function due to glycerol injection. This endpoint will apply if the mice behave ill, are not motile and/or are sitting apathetic in the cage.
3. Severe necrosis of muscle after glycerol injection. This endpoint will apply if necrosis becomes a visible lesion.

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Indicate the likely incidence.

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A knock-out phenotype could rarely lead to specific unexpected defects which may indicate a humane endpoint but this is very unlikely as it was until now not observed in these mice. The animal procedures can lead to experiment-related endpoints. These include wound infection after unilateral kidney ischemia reperfusion injury as well as severe necrosis after glycerol injection and a severe decrease of the kidney function which can happen due to overreaction against the injected glycerol. In our experience both wound inflammation and necrosis after glycerol injection are both very rare observation happening in less than 1% of the mice.

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#### **K. Classification of severity of procedures**

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Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned (non-recovery, mild, moderate, severe ).

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Based on the individual procedures listed below, we anticipate a cumulative level of discomfort that is mild for doxycycline treated non-injected mice (5%), mice injected with vehicle (3%) and moderate for mice that are either injected with glycerol or in which the ischemia/reperfusion model is induced (92%).

-Injection vehicle: mild

-Anaesthesia: moderate

-Injection of glycerol: moderate

-Ischemia/reperfusion: moderate

-Housing in metabolic cage: mild

-Blood collection (baseline): mild

- Sacrificing: mild

| Experiment                      | 1   |          |     | 2   |            |
|---------------------------------|-----|----------|-----|-----|------------|
| Group                           | Ctr | Glycerol | I/R | Ctr | Knock-outs |
| Nr. animals                     | 14  | 70       | 70  | 5   | 20         |
| Injection vehicle               | Yes | No       | No  | No  | No         |
| Anaesthesia                     | No  | No       | Yes | No  | No         |
| Injection of glycerol           | No  | Yes      | No  | No  | No         |
| Unilateral ischemia reperfusion | No  | No       | Yes | No  | No         |
| Housing in metabolic cage       | Yes | Yes      | Yes | No  | No         |

| Experiment                      | 3                       |                         |
|---------------------------------|-------------------------|-------------------------|
| Group                           | Disease model wild-type | Disease model Knock-out |
| Nr. animals                     | 64                      | 256                     |
| Injection vehicle               | No                      | No                      |
| Anaesthesia                     | No                      | No                      |
| Injection of glycerol           | Yes                     | Yes                     |
| Unilateral ischemia reperfusion | No                      | No                      |
| Housing in metabolic cage       | Yes                     | Yes                     |

## End of experiment

### L. Method of killing

Will the animals be killed during or after the procedures?

☐ No > Continue with Section 3: 'Signatures'.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

It is necessary to kill the animals at the end of the experiment to collect tissue needed for analysis and for data collection. Importantly, we need the kidneys of the mice and also we have to collect blood from the mice. Therefore, to avoid suffering from more animal procedures it is necessary to kill the animals at the end of the experiment.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☒ Yes

## DEC-advies

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### A. Algemene gegevens over de procedure

1. Aanvraagnummer: 2016-0029
2. Titel van het project: Resolving the cell-specific actions of Stromal Derived Factor 1 in kidney injury and regeneration
3. Titel van de NTS: Onderzoek naar nieuwe aanknopingspunten voor de ontwikkeling van behandelingen die nierschade kunnen beperken of genezen
4. Type aanvraag:
  - ☒ nieuwe aanvraag projectvergunning
  - ☐ wijziging van vergunning met nummer
5. Contactgegevens DEC:
  - naam DEC: RUDEC
  - telefoonnummer contactpersoon: [REDACTED], bereikbaar op maandag, dinsdag, en donderdag van 9:00 tot 15:00 uur
  - e-mailadres contactpersoon: [REDACTED]
6. Adviestraject (data dd-mm-jjjj):
  - ☒ ontvangen door DEC: 22-02-2017
  - ☐ aanvraag compleet
  - ☒ in vergadering besproken: 07-03-2017
  - ☐ anderszins behandeld
  - ☒ termijnonderbreking(en) van 13-3-2017 tot 11-04-2017
  - ☐ besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen
  - ☒ aanpassing aanvraag: 11-04-2017
  - ☒ advies aan CCD: 08-05-2017
7. De inhoud van dit project is afgestemd met de IvD en deze heeft geen bezwaren tegen de uitvoering van het project binnen deze instelling.
8. Eventueel horen van aanvrager: n.v.t.
9. Correspondentie met de aanvrager:
  - Datum vragen: 13-03-2017
  - Datum antwoorden: 11-04-2017
  - Gestelde vragen en antwoorden:

#### Project Proposal:

-2.1: De commissie is van mening dat dit geen translationeel maar uitsluitend basaal onderzoek betreft.

*Antwoord: Het project is nu gecategoriseerd als "basic research"*

-3.1: De eerste zin is te algemeen gesteld en kan beter weggelaten worden.

*Antwoord: Zoals aanbevolen hebben we de eerste zin verwijderd*

-3.2: In het project is sprake van (genummerde) aims, key objectives en key questions. De onderzoekers worden verzocht na te gaan of de verwijzingen naar de key questions en key objectives in alle onderdelen (inclusief figuren) van het project eenduidig, volledig en consequent is.

*Antwoord: We zijn dit nagegaan en de verwijzingen zijn consequent doorgevoerd.*

-3.2: Deze aanvraag spitst zich toe op SDF-1. In het geformuleerde hoofddoel van de aanvraag komt dit onvoldoende tot uiting.

*Antwoord: Om SDF-1 signaling meer in het hoofdoel te benadrukken hebben we de volgende zin aan het doel toegevoegd.*

*"In this proposal we will study the importance of SDF-1 signaling in the processes of kidney disease and repair."*

-3.3: Zijn er 1 miljoen patiënten die lijden aan nierschade? De onderzoekers worden verzocht dit iets genuanceerder te presenteren.

*Antwoord: We hebben deze zin veranderd en met nieuwe waarden die door de Nierstichting bekend zijn gemaakt aangevuld. De volgende zin is aan sectie 3.3. Relevance toegevoegd.*

*"In the Netherlands, 10% of the Dutch population has chronic kidney injury (1.7 million people). However, 40% of them (680,000 people) are not aware of this injury as complaints often only arise when the renal function has declined to 30%. People with chronic kidney injury have an increased risk of kidney failure, and death from cardiovascular disease."*

-3.4.2: Key questions 6 en 7 worden in de tekst van key objective 4 genoemd zonder uitleg wat hiermee wordt bedoeld. De onderzoekers worden verzocht dit te verhelderen.

*Antwoord: De verwijzingen naar de Key questions zijn uit de tekst verwijderd.*

-3.4.2: Uit de DAP blijkt dat om praktische redenen het adriamycine model gebruikt zal worden. Dit lijkt in tegenspraak met de bewering dat het beste model (adriamycine of Thy 1.1) gekozen zal worden om deze experimenten mee uit te voeren.

*Antwoord: We hebben de voorkeur voor het adriamycine model uit de aanvraag verwijderd en zullen werken met het model wat het beste werkt voor het onderzoek naar SDF-1 in glomerulosclerose.*

#### **Description of Animal Procedures:**

**\*DAP1**

-A1: aim 2, onderdeel 2.2: de vraagstelling van dit onderzoek is ziektegerelateerd. Waarom wordt de rol van SDF-1 signalling in de ontwikkeling van glomeruli en hun normale functie onderzocht? Wat is de plaats van aim 2 in de strategie van de projectaanvraag?

*Antwoord: In dit project wordt zoals in de research strategie de rol van SDF-1 onderzocht in zowel the normale fysiologie en pathofysiologie van de nier. Om te kunnen begrijpen hoe SDF-1 betrokken is bij het ziektebeeld moeten we ook begrijpen of en hoe SDF-1 betrokken is bij de normale fysiologie van de nier. Zeker als we uiteindelijk (buiten de scope van deze aanvraag) dieren willen behandelen met remmers voor SDF-1. Toekomstige behandeling zal enkel gedaan worden in volledig ontwikkelde nieren. Daarom hebben we het experiment waarin we de rol van SDF-1 in de ontwikkeling van de nier willen onderzoeken uit de aanvraag verwijderd.*

-J: Zijn er geen proefspecifieke humane eindpunten? Waardoor ontstaat de necrose aan de staart? Kan deze necrose niet vermeden worden door bijvoorbeeld een andere injectieroute te kiezen of hogere eisen te stellen aan de skills van de injecteur?

*Antwoord: We hebben het volgende proefspecifieke eindpunt toegevoegd:*

*1. Kidney failure or severe decrease of kidney function due to induction of glomerulosclerosis. This endpoint will apply if the mice become edemic, behave ill, are not motile and/or are sitting apathetic in the cage."*

*Wat betreft de necrose aan de staart geven we al aan dat we door de expertise van de biotechnisch personeel van het CDL geen problemen bij de injecties verwachten.*

-J3: De eerste zin is niet begrijpelijk voor de commissie. Er worden in dit project toch geen dieren gedood zonder voorafgaande handelingen?

*Antwoord: We hebben de zin veranderd. Er staat nu het volgende:*

*"The induction of glomerulosclerosis can lead to a reduced kidney function. In this case mice will become edemic. In regard to the disease models mentioned in this proposal we have observed this only in a few cases (less than 5%). Furthermore, a knock-out phenotype could rarely lead to specific unexpected defects which may indicate a humane endpoint but this is very unlikely as it was until now not observed in these mice."*

\*DAP2

-A1: In de dikgedrukte zin in de inleiding ontbreekt aim 2.

*Antwoord: Aim 2 is aan de zin toegevoegd.*

-J: De verwachte incidentie van de ernstige necrose en overreactie na glycerol injectie is niet met een percentage aangegeven.

*Antwoord: Dit hebben we veranderd. De incidentie wordt nu als volgt beschreven:*

*"The animal procedures can lead to experiment-related endpoints. These include wound infection after unilateral kidney ischemia reperfusion injury as well as severe necrosis after glycerol injection and a severe decrease of the kidney function which can happen due to overreaction against the injected glycerol. In our experience, both wound inflammation and necrosis after glycerol injection are both very rare observations happening in less than 1% of the mice."*

-K: Doden zonder voorafgaande handelingen is licht ongerief. Is er geen ongerief voor de dieren (4%) die alleen doxycycline via het drinkwater krijgen?

*Antwoord: Dit is aangepast*

-De onderzoekers worden verzocht na te gaan of de vragen en opmerkingen over één van beide DAPs ook van toepassing zijn op de andere DAP.

*Antwoord: Dit hebben we gedaan*

#### **Niet-technische samenvatting:**

-3.5: De commissie adviseert om het woord 'licht' in plaats van 'mild' te gebruiken.

*Antwoord: We hebben 'mild' vervangen voor 'licht'.*

-De onderzoekers worden verzocht te checken of de beantwoording van bovenstaande vragen over het Project Proposal en de DAPs ook leidt tot aanpassingen in de NTS.

*Antwoord: Door de aanpassingen die gemaakt zijn in de aanvraag is het totaal aantal dieren veranderd (nu 1086 ipv 1116 muizen) . Dit is in de DAP beschrijving en NTS aangepast.*

- De antwoorden hebben geleid tot aanpassing van de aanvraag

10. Eventuele adviezen door experts (niet lid van de DEC): n.v.t.

## **B. Beoordeling (adviesvraag en behandeling)**

1. Het project is vergunningplichtig (dierproeven in de zin der wet).
2. De aanvraag betreft een nieuwe aanvraag.
3. De DEC is competent om hierover te adviseren.
4. Er is geen betrokkenheid van DEC-leden bij deze projectaanvraag, waardoor onafhankelijkheid en onpartijdigheid zijn gewaarborgd.

## **C. Beoordeling (inhoud)**

1. Deze aanvraag heeft een concrete doelstelling en kan getypeerd worden als een project. De opzet komt het best overeen met voorbeeld 4B uit de handreiking 'Wat is een project'. De verschillende subdoelen zijn noodzakelijk om de doelstelling te behalen. Het is niet mogelijk om de individuele doelen te toetsen, omdat er sprake is van onderlinge afhankelijkheid. Het is helder welke handelingen individuele dieren zullen ondergaan. Hierdoor is ook duidelijk welk ongerief individuele dieren zullen ondergaan. De aanvrager heeft, zowel binnen de doelstellingen en bijlagen dierproeven, als tussen de doelstellingen, duidelijk beschreven op basis van welke criteria zij zal besluiten het project wel of niet te continueren. De DEC is er daardoor van overtuigd dat de aanvrager gedurende het project op zorgvuldige wijze besluiten zal nemen over de voortgang van het project en er niet onnodig dieren gebruikt zullen worden. In dit project wordt één gen bestudeerd in glomeruli en tubuli van de nier, waardoor de samenhang evident is. Gezien bovenstaande is de DEC van mening dat de aanvraag toetsbaar is en voldoende samenhang heeft.
2. Voor zover de DEC weet is er geen "tegenstrijdige" wetgeving die het uitvoeren van de experimenten in de weg zou kunnen staan.
3. De in de aanvraag aangekruiste doelcategorie is in overeenstemming met de hoofddoelstelling.

### *Belangen en waarden*

4. Het directe doel van het project is bij muismodellen het belang van SDF-1 signaling in de processen van nierziekte en herstel te bepalen. Het uiteindelijke doel is om een therapie te ontwikkelen waarmee nierschade kan worden beperkt of gerepareerd bij patiënten met een nierziekte. De onderzoekers focussen op het effect van SDF-1 en de beide receptoren voor dit chemokine: CXCR4 en CXCR7. Hiervoor worden state of the art induceerbare celspecifieke knockout muizenlijnen gebruikt waardoor de bijdrage van de verschillende componenten tijdens beschadiging van zowel glomeruli als tubuli apart onderzocht kan worden. Indien een nieuwe farmaceutische aanpak voor herstel of vermindering van nierschade wordt gevonden, dan zal de

klinische toepasbaarheid daarvan in vervolgonderzoek worden bepaald. Er is binnen dit project nog geen directe relatie tussen het doel van deze projectaanvraag en het uiteindelijke doel. De DEC acht het niet waarschijnlijk dat het uiteindelijke doel behaald zal worden binnen de duur van dit project. De aanvrager heeft duidelijk gemaakt wat de status van het onderzoeksveld is en wat de bijdrage van dit project daaraan zal zijn. Uit de aanvraag blijkt dat de kennis van de bijdrage van de SDF1/CXCR4/CXCR7 as aan pathofysiologische processen bij nierziekten op dit moment zeer beperkt is, dat deze kennis kan bijdragen aan het ontwikkelen van nieuwe behandelmogelijkheden van progressieve nierziekten en dat daar behoefte aan is. Naar de mening van de DEC is het doel van deze projectaanvraag daarom gerechtvaardigd binnen de context van het onderzoeksveld.

5. De belangrijkste belanghebbenden in deze projectaanvraag zijn de proefdieren, de onderzoekers en de doelgroep/patiënten.

Voor de proefdieren geldt dat hun welzijn en integriteit worden aangetast. De dieren zullen beperkt worden in hun natuurlijke gedrag en gedurende de proeven zullen de dieren stress ondervinden en pijn ondergaan. Uiteindelijk zullen ze in het kader van het onderzoek gedood worden. De dieren hebben er belang bij hiervan gevrijwaard te blijven.

Voor de onderzoekers geldt dat het publiceren van belangrijke nieuwe wetenschappelijke inzichten resulteert in een goede wetenschappelijke reputatie, hetgeen vaak de sleutel is voor het verkrijgen van nieuwe onderzoeksmiddelen en mogelijkheden. Dit kan door de onderzoeker zelf van belang geacht worden, maar dient naar de mening van de DEC geen rol te spelen in de ethische afweging over de toelaatbaarheid van het gebruik van proefdieren. Het gaat uiteindelijk om de vraag of dit onderzoek belangrijke maatschappelijke en wetenschappelijke doelen dient (gezondheid, kennis).

Voor patiënten is dit onderzoek indirect van belang, omdat het kan bijdragen aan een verbetering van hun gezondheid en kwaliteit van leven. Meer kennis over SDF-1signalering bij nierziekte en herstel kan leiden tot de ontwikkeling van nieuwe behandelmogelijkheden voor progressieve nierziekten. Nieuwe behandelingen kunnen er toe leiden dat de patiënt weer gezond wordt, dan wel een betere kwaliteit van leven heeft. Kunnen beschikken over adequate behandelingen voor ernstige ziekten, zoals chronische (progressieve) nierziekten, is van groot belang voor de samenleving.

6. Er is geen aanleiding voor de DEC om de bewering van de aanvrager dat er geen nadelige effecten voor het milieu te verwachten zijn, in twijfel te trekken.

#### *Proefopzet en haalbaarheid*

7. De kennis en kunde van de onderzoeksgroep en andere betrokkenen bij de dierproeven zijn voldoende gewaarborgd. De commissie is overtuigd van de kwaliteit van het werk van de aanvrager, zoals blijkt uit de in de aanvraag vermelde publicaties van deze onderzoeksgroep. De aanvragers beschikken over voldoende kennis en kunde om te kunnen voldoen aan alle zorgvuldigheidseisen omtrent het verrichten van dierproeven.
8. De doelstellingen van het project zijn realistisch en de voorgestelde experimentele opzet en uitkomstparameters sluiten hier logisch bij aan. Bovendien heeft deze groep veel ervaring in dit onderzoeksveld en met de voorgestelde dierproeven. De DEC is dan ook van mening dat het project goed is opgezet, en dat deze strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling binnen het kader van het project.

#### *Welzijn dieren*

9. Er is geen sprake van de volgende bijzonderheden op het gebied van categorieën van dieren, omstandigheden of behandeling van de dieren:
  - ☐ Bedreigde diersoort(en) (10e lid 4)
  - ☐ Niet-menselijke primaten (10e)
  - ☐ Dieren in/uit het wild (10f)
  - ☐ Niet gefokt voor dierproeven (11, bijlage I richtlijn)
  - ☐ Zwerfdieren (10h)
  - ☐ Hergebruik (1e lid 2)
  - ☐ Locatie: buiten instelling vergunninghouder (10g)
  - ☐ Geen toepassing verdoving/pijnbestrijding (13)
  - ☐ Dodingsmethode niet volgens bijlage IV richtlijn (13c lid 3)
10. De huisvesting en verzorging van de dieren zijn conform de eisen in bijlage III van richtlijn 2010/63/EU.
11. Het cumulatieve ongerief als gevolg van de dierproeven is realistisch ingeschat en geclassificeerd. Voor 42 % van de dieren wordt matig ongerief verwacht vanwege een operatie of door het inspuiten van glycerol in een pootspier. Voor de overige dieren (58%) wordt licht ongerief verwacht.
12. De integriteit van dieren wordt aangetast door genetische modificatie. Bij de meeste dieren worden deze modificaties alleen actief na toediening van een bepaalde stof en uitsluitend in bepaalde niercellen. Naar de mening van de DEC wordt de integriteit van de dieren daardoor niet noemenswaardig aangetast.
13. De criteria voor humane eindpunten zijn voldoende specifiek gedefinieerd en toegesneden op het experiment. Het percentage dieren dat naar verwachting een humaan eindpunt zal bereiken is op basis van ervaring met de gebruikte muismodellen en procedures ingeschat. De commissie is het eens met deze inschatting en de gehanteerde humane eindpunten.

3V's

14. De aanvrager heeft voldoende aannemelijk gemaakt dat er geen geschikte vervangingsalternatieven zijn. De aanvragers zullen de rol van SDF-1 in normale en pathologische omstandigheden eerst *in vitro* bestuderen bij gekweekte niercellen. De informatie hieruit zal gebruikt worden bij het design van de *in vivo* experimenten. Een nier is een complex orgaan dat uit meerdere celtypes bestaat en in samenhang met andere organen en de bloedsomloop functioneert. Nierziekten en het beloop daarvan kunnen dan ook niet goed met proefdier vrije alternatieven onderzocht worden. Een aantal onderdelen van het project zal dus *in vitro* bestudeerd kunnen worden. Voor het beantwoorden van de resterende onderzoeksvragen zijn dierproeven noodzakelijk.
15. Het maximale aantal te gebruiken dieren is realistisch ingeschat en is proportioneel ten opzichte van de gekozen onderzoekszet en de looptijd. De onderzoekers hanteren een goede strategie om ervoor te zorgen dat er met het kleinst mogelijke aantal dieren wordt gewerkt waarmee nog een wetenschappelijk betrouwbaar resultaat kan worden verkregen. Door de stapsgewijze aanpak waarin de resultaten uit eerdere proeven worden gebruikt voor het design van vervollexperimenten wordt onnodig gebruik van proefdieren voorkomen. Waar mogelijk worden

experimenten gecombineerd om optimaal gebruik te kunnen maken van het materiaal.

16. Het project is in overeenstemming met de vereiste van de verfijning van dierproeven. Muizen zijn de minst complexe diersoort waarin de ontwikkeling en functie van de nieren voldoende overeenkomt met de menselijke nier, en waarvoor onderzoeksmethoden beschikbaar zijn om dit onderzoek uit te kunnen voeren. Het gebruik van metabole kooien wordt zoveel mogelijk beperkt. De DEC is ervan overtuigd dat de beschreven proefopzet de meest verfijnde is en dat de dierproeven zo humaan mogelijk worden uitgevoerd.
17. Het betreft geen wettelijk vereist onderzoek.

*Dieren in voorraad gedood en bestemming dieren na afloop proef*

18. Dieren van beide geslachten zullen ingezet worden.
19. De dieren zullen in het kader van het project gedood worden. Dit is noodzakelijk om verschillende weefsels te kunnen onderzoeken voor het beantwoorden van bepaalde onderzoeksvragen. De gebruikte dodingsmethode staat vermeld in bijlage IV van richtlijn 2010/63/EU.
20. Er worden in deze projectaanvraag geen landbouwhuisdieren, honden, katten of niet-humane primaten gebruikt (en dus ook niet gedood om niet-wetenschappelijke redenen).

*NTS*

21. De niet-technische samenvatting is een evenwichtige weergave van het project en begrijpelijk geformuleerd.

**D. Ethische afweging**

1. Rechtvaardigt het belang van de doelstelling van het project het ongerief dat de dieren wordt aangedaan, en is aan alle zorgvuldigheidseisen (3V's) voldaan?
2. Er vindt een lichte of matige aantasting van welzijn en integriteit van de proefdieren plaats (beschreven in C9 tot C20). De doelstellingen kunnen niet zonder dieren behaald worden. De onderzoekers doen al het mogelijke om het lijden van de dieren en het aantal dieren te beperken.  
Voor patiënten is dit onderzoek van belang, omdat het kan bijdragen aan een verbetering van hun geestelijke gezondheid en kwaliteit van leven. De DEC kent daar veel gewicht aan toe om de volgende redenen. Chronische nierziekte (CKD) komt vaak voor: 10% van de Nederlanders heeft CKD, waardoor zij een grotere kans hebben op nierfalen en sterfte door cardiovasculaire ziekte. Ernstig nierfalen kan alleen behandeld worden met niervervangende therapie. Meer kennis van de processen die zich afspelen tijdens chronische progressieve nierziekte kunnen bijdragen aan de ontwikkeling van nieuwe therapieën om het degeneratieve proces te stoppen en herstel te bevorderen. Het is aannemelijk dat de doelstellingen op termijn behaald zullen worden. De commissie acht het vergaren van meer kennis over de processen die zich afspelen tijdens CKD van substantieel belang.
3. De DEC is overtuigd van het belang van de doelstellingen: bij muismodellen het belang van SDF-1 signalering in de processen van nierziekte en herstel te bepalen. Het uiteindelijke doel is om een

therapie te ontwikkelen waarmee nierschade kan worden beperkt of gerepareerd bij patiënten met een nierziekte. De DEC is van mening dat de belangen van de patiënten voldoende zwaar wegen om het schaden van de belangen van de proefdieren (om gevrijwaard te blijven van een aantasting van hun welzijn en integriteit) te rechtvaardigen. De commissie is overtuigd van de kwaliteit van het werk van de aanvrager. De DEC is van mening dat het project goed is opgezet, en dat de gekozen strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling binnen het kader van het project. De aanvrager heeft voldoende aannemelijk gemaakt dat er geen geschikte vervangingsalternatieven zijn, dat het doel niet met minder dieren behaald kan worden, dat de gebruikte aanpak de meest verfijnde is en dat zij zal kunnen voorkomen dat mens, dier en het milieu onbedoelde negatieve effecten ondervinden als gevolg van de dierproeven.

De DEC is van oordeel dat het hier boven geschetste belang de onvermijdelijke nadelige gevolgen van dit onderzoek voor de dieren, in de vorm van angst, pijn of stress, rechtvaardigt. Aan de eis dat het belang van de experimenten op dient te wegen tegen het ongerief dat de dieren wordt berokkend, is voldaan.

## E. Advies

### 1. Advies aan de CCD

☒ De DEC adviseert de vergunning te verlenen

☐ De DEC adviseert de vergunning te verlenen onder de volgende voorwaarden

- Op grond van het wettelijk vereiste dient de projectleider bij beëindiging van het project een beoordeling achteraf aan te leveren die is afgestemd met de IvD.
- Voor de uitvoering van dit project is tevens ministeriële ontheffing vereist
- Overige door de DEC aan de uitvoering verbonden voorwaarden, te weten...

☐ De DEC adviseert de vergunning niet te verlenen vanwege:

- De vaststelling dat het project niet vergunningplichtig is om de volgende redenen:...
- De volgende doorslaggevende ethische bezwaren:...
- De volgende tekortkomingen in de aanvraag:...

### 2. Het uitgebrachte advies is gebaseerd op consensus.

### 3. Er zijn geen knelpunten of dilemma's geconstateerd – zowel binnen als buiten de context van het project - die de verantwoordelijkheid en competentie van de DEC overstijgen.



> Retouradres Postbus 20401 2500 EK Den Haag

Stichting Katholieke Universiteit Nijmegen



**Centrale Commissie  
Dierproeven**

Postbus 20401  
2500 EK Den Haag  
centralecommissiedierproeven.nl  
0900 28 000 28 (10 ct/min)  
info@zbo-ccd.nl

**Onze referentie**

Aanvraagnummer  
AVD1030020171674

**Bijlagen**

2

Datum 8 mei 2017

Betreft Ontvangstbevestiging aanvraag projectvergunning Dierproeven

Geachte [REDACTED],

Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 8 mei 2017. Het gaat om uw project "Resolving the cell-specific actions of SDF-1 in kidney injury and regeneration". Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD1030020171674. Gebruik dit nummer wanneer u contact met de CCD opneemt.

**Wacht met de uitvoering van uw project**

Als wij nog informatie van u nodig hebben dan ontvangt u daarover bericht. Uw aanvraag is in ieder geval niet compleet als de leges niet zijn bijgeschreven op de rekening van de CCD. U ontvangt binnen veertig werkdagen een beslissing op uw aanvraag. Als wij nog informatie van u nodig hebben, wordt deze termijn opgeschort. In geval van een complexe aanvraag kan deze termijn met maximaal vijftien werkdagen verlengd worden. U krijgt bericht als de beslisperiode van uw aanvraag vanwege complexiteit wordt verlengd. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project.

**Factuur**

Bijgaand treft u de factuur aan voor de betaling van de leges. Wij verzoeken u de leges zo spoedig mogelijk te voldoen, zodat we uw aanvraag in behandeling kunnen nemen. Is uw betaling niet binnen dertig dagen ontvangen, dan kan uw aanvraag buiten behandeling worden gesteld. Dit betekent dat uw aanvraag niet beoordeeld wordt en u uw project niet mag starten.

**Meer informatie**

Heeft u vragen, kijk dan op [www.centralecommissiedierproeven.nl](http://www.centralecommissiedierproeven.nl). Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:

- Gegevens aanvraagformulier
- Factuur

**Datum:**

8 mei 2017

**Aanvraagnummer:**

AVD1030020171674

**Datum:**  
8 mei 2017  
**Aanvraagnummer:**  
AVD1030020171674

### **Gegevens aanvrager**

Uw gegevens

Deelnemersnummer NVWA: 10300  
Naam instelling of organisatie: Stichting Katholieke Universiteit Nijmegen  
Naam portefeuillehouder of  
diens gemachtigde: [REDACTED]  
KvK-nummer: 41055629  
Straat en huisnummer: [REDACTED]  
Postbus: [REDACTED]  
Postcode en plaats: [REDACTED]  
IBAN: NL90ABNA0231209983  
Tenaamstelling van het  
rekeningnummer: UMC St Radboud

Gegevens verantwoordelijke onderzoeker

Naam: [REDACTED]  
Functie: Universitair docent/Onderzoeker  
Afdeling: [REDACTED]  
Telefoonnummer: [REDACTED]  
E-mailadres: [REDACTED]

**Datum:**  
8 mei 2017  
**Aanvraagnummer:**  
AVD1030020171674

Gegevens verantwoordelijke uitvoering proces

Naam: [REDACTED]  
Functie: Instantievoor Dierenwelzijn  
Afdeling: [REDACTED]  
Telefoonnummer: [REDACTED]  
E-mailadres: [REDACTED]

**Over uw aanvraag**

Wat voor aanvraag doet u? ☒ Nieuwe aanvraag  
☐ Wijziging op een (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn  
☐ Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn

**Over uw project**

Geplande startdatum: 1 augustus 2017  
Geplande einddatum: 1 augustus 2022  
Titel project: Resolving the cell-specific actions of SDF-1 in kidney injury and regeneration  
Titel niet-technische samenvatting: Onderzoek naar nieuwe aanknopingspunten voor de ontwikkeling van behandelingen etc  
Naam DEC: RU DEC  
Postadres DEC: [REDACTED]  
E-mailadres DEC: [REDACTED]

**Betaalgegevens**

De leges bedragen: € 1.287,-  
De leges voldoet u: na ontvangst van de factuur

**Checklist bijlagen**

Verplichte bijlagen: ☒ Projectvoorstel  
☒ Beschrijving Dierproeven  
☒ Niet-technische samenvatting  
Overige bijlagen: ☒ DEC-advies

**Ondertekening**

Naam:

Functie:

Plaats:

Datum:



Instantie voor dierenwelzijn

Nijmegen

8 mei 2017

**Datum:**

8 mei 2017

**Aanvraagnummer:**

AVD1030020171674



> Retouradres Postbus 20401 2500 EK Den Haag

Geert Grooteplein 29  
Postbus 9101, HP 231, route 231 (CDL)  
6500 HB NIJMEGEN



**Centrale Commissie  
Dierproeven**

Postbus 20401  
2500 EK Den Haag  
centralecommissiedierproeven.nl  
0900 28 000 28 (10 ct/min)  
info@zbo-ccd.nl

**Onze referentie**

Aanvraagnummer  
AVD1030020171674

**Bijlagen**

2

Datum 8 mei 2017  
Betreft Factuur aanvraag projectvergunning Dierproeven

**Factuur**

Factuurdatum: 8 mei 2017

Vervaldatum: 7 juni 2017

Factuurnummer: 171674

Ordernummer: Kostenplaats en kostensoort: 040823-461220 CDL

projectnummer: 2016-0029 Verantwoordelijk onderzoeker: [REDACTED]

| Omschrijving                                                                      | Bedrag     |
|-----------------------------------------------------------------------------------|------------|
| Betaling leges projectvergunning dierproeven<br>Betreft aanvraag AVD1030020171674 | € 1.287,00 |

Wij verzoeken u het totaalbedrag vóór de gestelde vervaldatum over te maken op rekening NL29INGB 070.500.1512 onder vermelding van het factuurnummer en aanvraagnummer, ten name van Centrale Commissie Dierproeven, Postbus 93144, 2509 AC te 's Gravenhage.

**Van:** [REDACTED]  
**Verzonden:** woensdag 31 mei 2017 9:55  
**Aan:** info@zbo-ccd.nl  
**CC:** [REDACTED]  
**Onderwerp:** RE: vraag bij de behandeling van AVD1030020171674  
**Bijlagen:** AVD1030020171674-nieuweNTS\_RUDEC2016-0029.pdf  
**Categorieën:** Dossier: [REDACTED]

Antwoord op uw e-mail van 23 mei 2017 (zie onderstaand), hierbij verstuurd door de IvD van Radboudumc/RU namens de onderzoeker [REDACTED].

Als bijlage de aangepaste NTS. Indien het nodig dat we deze uploaden via de beschermde NetFTP-verbinding dan zullen we dat ook doen (laat u dat weten svp?).

mvg, [REDACTED]

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Nijmegen, 31 mei 2017

Geachte mevrouw [REDACTED]

We hebben de niet technische samenvatting van het project, "Resolving the cell-specific actions of SDF-1 in kidney injury and regeneration" met aanvraag nummer AVD1030020171674, zoals voorgesteld aangepast. De beschrijving van de handeling 'uit de proef genomen worden' is nu vervangen door 'worden gedood'.

Met vriendelijke groet,

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

The Netherlands

[www.radboudumc.nl](http://www.radboudumc.nl)

---

**Van:** Info-zbo [<mailto:info@zbo-ccd.nl>]

**Verzonden:** dinsdag 23 mei 2017 14:56

**Aan:** [REDACTED]

**CC:** Postbus instantie voor dierenwelzijn

**Onderwerp:** vraag bij de behandeling van AVD1030020171674

Geachte [REDACTED],

U heeft bij de CCD een aanvraag tot projectvergunning ingediend. Bij de behandeling hiervan zouden wij u willen verzoeken een aanpassing in de Niet technische samenvatting te doen. Het betreft uw project 'Resolving the cell-specific actions of SDF-1 in kidney injury and regeneration' met aanvraag nummer AVD1030020171674.

U beschrijft in de NTS onder punt 4.4 dat de dieren bij het bereiken van de Humane Eindpunten ‘uit de proef genomen worden’. De CCD hecht aan een transparante communicatie richting het publiek, en als zodanig is de NTS ook bedoeld, en ziet dit als verhullend taalgebruik. Wij willen u dan ook verzoeken om deze zin aan te passen naar bijvoorbeeld: ‘bij het bereiken van de Humane eindpunten worden de dieren gedood’

Uw aanvraag zal in de vergadering van 2 juni door de CCD besproken worden,

Vriendelijke groet, [REDACTED]

Namens **Centrale Commissie Dierproeven**

[www.centralecommissiedierproeven.nl](http://www.centralecommissiedierproeven.nl)

.....

**Postbus 20401 | 2500 EK | Den Haag**

.....

T: 0900 2800028

E: [info@zbo-ccd.nl](mailto:info@zbo-ccd.nl) (let op: nieuw emailadres!)

Het Radboudumc staat geregistreerd bij de Kamer van Koophandel in het handelsregister onder nummer 41055629. The Radboud university medical center is listed in the Commercial Register of the Chamber of Commerce under file number 41055629.



> Retouradres Postbus 20401 2500 EK Den Haag

Stichting Katholieke Universiteit Nijmegen

Postbus 9101,

6500 HB NIJMEGEN



**Centrale Commissie  
Dierproeven**

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centralecommissiedierproeven.nl

0900 28 000 28 (10 ct/min)

info@zbo-ccd.nl

**Onze referentie**

Aanvraagnummer

AVD1030020171674

**Bijlagen**

1

Datum 6 juni 2017

Betreft Beslissing aanvraag projectvergunning Dierproeven

Geachte [REDACTED],

Op 8 mei 2017 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Resolving the cell-specific actions of SDF-1 in kidney injury and regeneration" met aanvraagnummer AVD1030020171674. Wij hebben uw aanvraag beoordeeld.

Op 31 mei 2017 heeft u uw aanvraag aangevuld. Op ons verzoek heeft u de Niet Technische samenvatting gewijzigd.

### **Beslissing**

Wij keuren uw aanvraag goed op grond van artikel 10a van de Wet op de Dierproeven (hierna: de wet). Hierbij gelden de voorwaarden zoals genoemd in de vergunning.

Met het oog op artikel 10a, lid 1, zijn er algemene voorwaarden gesteld.

U kunt met uw project "Resolving the cell-specific actions of SDF-1 in kidney injury and regeneration" starten. De vergunning wordt afgegeven van 1 augustus 2017 tot en met 31 juli 2022. Deze termijn is anders dan in uw aanvraag, omdat de looptijd van een vergunning niet langer kan zijn dan 5 jaar.

Overige wettelijke bepalingen blijven van kracht.

### **Procedure**

Bij uw aanvraag heeft u een advies van de Dierexperimentencommissie RU DEC gevoegd. Dit advies is opgesteld op 8 mei 2017. Bij de beoordeling van uw aanvraag is dit advies betrokken overeenkomstig artikel 10a, lid 3 van de wet.

Wij kunnen ons vinden in de inhoud van het advies van de Dierexperimentencommissie. Dit advies van de commissie nemen wij over, inclusief de daaraan ten grondslag liggende motivering. Er worden aanvullende algemene voorwaarde(n) gesteld. Het DEC-advies en de in de bijlage opgenomen beschrijving van de artikelen van de wet- en regelgeving zijn de grondslag van dit besluit.

**Datum:**  
6 juni 2017  
**Aanvraagnummer:**  
AVD1030020171674

#### **Bezwaar**

Als u het niet eens bent met deze beslissing, kunt u binnen zes weken na verzending van deze brief schriftelijk een bezwaarschrift indienen. Een bezwaarschrift kunt u sturen naar Centrale Commissie Dierproeven, afdeling Juridische Zaken, postbus 20401, 2500 EK Den Haag.

Bij het indienen van een bezwaarschrift vragen we u in ieder geval de datum van de beslissing waartegen u bezwaar maakt en het aanvraagnummer te vermelden. U vindt deze nummers in de rechter kantlijn in deze brief.

Bezwaar schorst niet de werking van het besluit waar u het niet mee eens bent. Dat betekent dat dat besluit wel in werking treedt en geldig is. U kunt tijdens deze procedure een voorlopige voorziening vragen bij de Voorzieningenrechter van de rechtbank in de woonplaats van de aanvrager. U moet dan wel kunnen aantonen dat er sprake is van een spoedeisend belang.

Voor de behandeling van een voorlopige voorziening is griffierecht verschuldigd. Op <http://www.rechtspraak.nl/Organisatie/Rechtbanken/Pages/default.aspx> kunt u zien onder welke rechtbank de vestigingsplaats van de aanvrager valt.

#### **Meer informatie**

Heeft u vragen, kijk dan op [www.centralecommissiedierproeven.nl](http://www.centralecommissiedierproeven.nl). Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Centrale Commissie Dierproeven  
namens deze:

  
M. G. de Putter  
Algemeen Secretaris

#### **Bijlagen:**

- Vergunning
- Hiervan deel uitmakend:
  - DEC-advies
  - Weergave wet- en regelgeving



# Projectvergunning

gelet op artikel 10a van de Wet op de Dierproeven

Verleent de Centrale Commissie Dierproeven aan

Naam: Stichting Katholieke Universiteit Nijmegen

Adres: Postbus 9101, t.a.v. [REDACTED]

Postcode en plaats: 6500 HB NIJMEGEN

Deelnemersnummer: 10300

deze projectvergunning voor het tijdvak 1 augustus 2017 tot en met 31 juli 2022, voor het project "Resolving the cell-specific actions of SDF-1 in kidney injury and regeneration" met aanvraagnummer AVD1030020171674, volgens advies van Dierexperimentencommissie RU DEC. Er worden aanvullende algemene voorwaarde(n) gesteld.

De functie van de verantwoordelijk onderzoeker is Universitair docent/Onderzoeker. Voor de uitvoering van het project is Instantievoor Dierenwelzijn verantwoordelijk.

De aanvraag omvat de volgende bescheiden:

- 1 een aanvraagformulier projectvergunning dierproeven, ontvangen op 8 mei 2017
- 2 de bij het aanvraagformulier behorende bijlagen:
  - a Projectvoorstel, zoals ontvangen per digitale indiening op 8 mei 2017;
  - b Niet-technische Samenvatting van het project, zoals ontvangen per digitale indiening op 31 mei 2017;
  - c Advies van dierexperimentencommissie d.d. 8 mei 2017, ontvangen op 8 mei 2017.
  - d De aanvullingen op uw aanvraag, ontvangen op 31 mei 2017

| Naam proef                                                         | Diersoort/ Stam         | Aantal dieren | Ernst                       | Opmerkingen |
|--------------------------------------------------------------------|-------------------------|---------------|-----------------------------|-------------|
| <b>3.4.4.1 SDF-1 signaling in glomerulosclerosis</b>               |                         |               |                             |             |
|                                                                    | Muizen (Mus musculus) / | 587           | 100%<br>Licht               |             |
| <b>3.4.4.2 SDF-1 signalling in tubular injury and regeneration</b> |                         |               |                             |             |
|                                                                    | Muizen (Mus musculus) / | 499           | 92%<br>Matig<br>8%<br>Licht |             |

## Voorwaarden

Op grond van artikel 10a1 lid 2 van de Wet op de dierproeven zijn aan een projectvergunning voorwaarden te stellen

**Aanvraagnummer:**  
AVD1030020171674

De vergunning wordt verleend onder de voorwaarde dat go/no go momenten worden afgestemd met de IvD.

In artikel 10, lid 1 sub a van de wet, wordt bepaald dat het verboden is een dierproef te verrichten voor een doel dat, naar de algemeen kenbare, onder deskundigen heersende opvatting, ook kan worden bereikt anders dan door middel van een dierproef, of door middel van een dierproef waarbij minder dieren kunnen worden gebruikt of minder ongerief wordt berokkend dan bij de in het geding zijnde proef het geval is. Nieuwe onderzoeken naar alternatieven kunnen tot gevolg hebben dat inzichten en/of omstandigheden van het aangevraagde project in de vergunningsperiode wijzigen, gedurende de looptijd van deze vergunning. Indien bovenstaande zich voordoet dient aanvrager dit in afstemming met de IvD te melden bij de CCD. De CCD kan in een dergelijke situatie aan de vergunning nieuwe voorwaarden verbinden en gestelde voorwaarde wijzigen of intrekken.



**Aanvraagnummer:**

AVD1030020171674

## Weergave wet- en regelgeving

### **Dit project en wijzigingen**

Volgens artikel 10c van de Wet op de Dierproeven (hierna de wet) is het verboden om andere dierproeven uit te voeren dan waar de vergunning voor is verleend. De dierproeven mogen slechts worden verricht in het kader van een project, volgens artikel 10g. Uit artikel 10b volgt dat de dierproeven zijn ingedeeld in de categorieën terminaal, licht, matig of ernstig. Als er wijzigingen in een dierproef plaatsvinden, moeten deze gemeld worden aan de Centrale Commissie Dierproeven. Hebben de wijzigingen negatieve gevolgen voor het dierenwelzijn, dan moet volgens artikel 10a5 de wijziging eerst voorgelegd worden en mag deze pas doorgevoerd worden na goedkeuren door de Centrale Commissie Dierproeven.

Artikel 10b schrijft voor dat het verboden is een dierproef te verrichten die leidt tot ernstige mate van pijn, lijden, angst of blijvende schade die waarschijnlijk langdurig zal zijn en niet kan worden verzacht, tenzij hiervoor door de Minister een ontheffing is verleend.

### **Verzorging**

De fokker, leverancier en gebruiker moeten volgens artikel 13f van de wet over voldoende personeel beschikken en ervoor zorgen dat de dieren behoorlijk worden verzorgd, behandeld en gehuisvest. Er moeten ook personen zijn die toezicht houden op het welzijn en de verzorging van de dieren in de inrichting, personeel dat met de dieren omgaat moet toegang hebben tot informatie over de in de inrichting gehuisveste soorten en personeel moet voldoende geschoold en bekwaam zijn. Ook moeten er personen zijn die een eind kunnen maken aan onnodige pijn, lijden, angst of blijvende schade die tijdens een dierproef bij een dier wordt veroorzaakt. Daarnaast zijn er personen die zorgen dat een project volgens deze vergunning wordt uitgevoerd en als dat niet mogelijk is zorgen dat er passende maatregelen worden getroffen.

In artikel 9 staat dat de persoon die het project en de dierproef opzet deskundig en bekwaam moet zijn. In artikel 8 van het Dierproevenbesluit 2014 staat dat personen die dierproeven verrichten, de dieren verzorgen of de dieren doden, hiervoor een opleiding moeten hebben afgerond.

Voordat een dierproef die onderdeel uitmaakt van dit project start, moet volgens artikel 10a3 van de wet de uitvoering afgestemd worden met de instantie voor dierenwelzijn.

### **Pijnbestrijding en verdoving**

In artikel 13 van de wet staat dat een dierproef onder algehele of plaatselijke verdoving wordt uitgevoerd tenzij dat niet mogelijk is, dan wel bij het verrichten van een dierproef worden pijnstillers toegediend of andere goede methoden gebruikt die de pijn, het lijden, de angst of de blijvende schade bij het dier tot een minimum beperken. Een dierproef die bij het dier gepaard gaat met zwaar letsel dat hevige pijn kan veroorzaken, wordt niet zonder verdoving uitgevoerd. Hierbij wordt afgewogen of het toedienen van verdoving voor het dier traumatischer is dan de dierproef zelf en het toedienen van verdoving onverenigbaar is met het doel van de dierproef. Bij een dier wordt geen stof toegediend waardoor het dier niet meer of slechts in verminderde mate in staat is pijn te tonen, wanneer het dier niet tegelijkertijd voldoende verdoving of pijnstilling krijgt toegediend, tenzij wetenschappelijk gemotiveerd. Dieren die pijn

**Aanvraagnummer:**

AVD1030020171674

kunnen lijden als de verdoving eenmaal is uitgewerkt, moeten preventief en postoperatief behandeld worden met pijnstillers of andere geschikte pijnbestrijdingsmethoden, mits die verenigbaar zijn met het doel van de dierproef. Zodra het doel van de dierproef is bereikt, moeten passende maatregelen worden genomen om het lijden van het dier tot een minimum te beperken.

**Einde van een dierproef**

Artikel 13a van de wet bepaalt dat een dierproef is afgelopen wanneer voor die dierproef geen verdere waarnemingen hoeven te worden verricht of, voor wat betreft nieuwe genetisch gemodificeerde dierenlijnen, wanneer bij de nakomelingen niet evenveel of meer, pijn, lijden, angst, of blijvende schade wordt waargenomen of verwacht dan bij het inbrengen van een naald. Er wordt dan door een dierenarts of een andere ter zake deskundige beslist of het dier in leven zal worden gehouden. Een dier wordt gedood als aannemelijk is dat het een matige of ernstige vorm van pijn, lijden, angst of blijvende schade zal blijven ondervinden. Als een dier in leven wordt gehouden, krijgt het de verzorging en huisvesting die past bij zijn gezondheidstoestand.

Volgens artikel 13b moet de dood als eindpunt van een dierproef zoveel mogelijk worden vermeden en vervangen door in een vroege fase vaststelbare, humane eindpunten. Als de dood als eindpunt onvermijdelijk is, moeten er zo weinig mogelijk dieren sterven en het lijden zo veel mogelijk beperkt blijven.

Uit artikel 13d volgt dat het doden van dieren door een deskundig persoon moet worden gedaan, wat zo min mogelijk pijn, lijden en angst met zich meebrengt. De methode om te doden is vastgesteld in de Europese richtlijn artikel 6.

In artikel 13c is vastgesteld dat proefdieren geadopteerd kunnen worden, teruggeplaatst in hun habitat of in een geschikt dierhouderijsysteem, als de gezondheidstoestand van het dier het toelaat, er geen gevaar is voor volksgezondheid, diergezondheid of milieu en er passende maatregelen zijn genomen om het welzijn van het dier te waarborgen.