Centrale Commissie Dierproeven

# 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 on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0800-7890789).

## 1 General information

| | | |
|---|---|---|
| 1.1 | Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'. | |
| 1.2 | Provide the name of the licenced establishment. | Biomedical Primate Research Centre |
| 1.3 | List the serial number and type of animal procedure<br><br>*Use the numbers provided at 3.4.3 of the project proposal.* | Serial number: 2<br>Type of animal procedure: Intravenous injection of antibodies for **brain PK** evaluation. |

## 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 primary aim of this experiment is to establish brain PK of the blood-brain barrier (BBB) shuttle antibody, TXP1. These results will be thereafter used to model and identify the most optimal dosing strategy for clinical translation.

The animals will be injected i.v. with TXP1 or isotype control antibody (G12) at a defined concentration based upon data from our previous study. At various time-points , 2 animals per compound will be euthanised. The selected timepoints will be day 1 after injection (comparable to the previous study), the second timepoint is envisaged at day 5 after injection. Depending on the data and only when needed to obtain a robust data set a third timepoint will be added retrospectively.

Additional blood samples might be obtained at several timepoints for comparison with the blood PK data obtained in appendix 1.

Brain and other peripheral organs will be collected alongside blood and CSF for ELISA-based concentration assessment. The use of comparator antibody (G12) with no target binding ability would allow us to correctly identify PK parameter that include brain half-life ($T_{1/2}$ - brain), brain saturation profile (Time to $C_{max}$), brain distribution, receptor saturation point, dose-dependent brain exposure (AUC – brain).

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

Animals will be intravenously (IV) injected at saphenous vein with the compound and the negative control under sedation.

The animals will be euthanised after cardiac perfusion at specific timepoints. Organs will be collected post-mortem for further analysis, including pathology if needed.

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

In PK studies, each animal is its own control. The number of animals is based upon earlier results with comparable compounds obtained in animal studies. PK profiles are usually relatively comparable between individuals and therefore the number of animals per group can be limited. Based upon our earlier results 2 animals per group are sufficient in this study to obtain reliable results.

A maximum number of 12 animals is anticipated for the full brain PK study, using two animals per group., two compounds and three timepoints. The third timepoint will only be included when needed based upon the obtained results.

Based on our previous experience from NHP and rodent PK studies we expect the variability level of 6% CV. Consequently, it justifies the use of two animals per group per compound. The data generated from these experiments are expected to be of sufficiently low variability to generate statistically significant output while using a minimal number of animals.

**B. The animals**

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

| Serial number | Species | Origin | Life stages | Number | Gender | Genetically altered | Strain |
|---|---|---|---|---|---|---|---|
| 2 | macaca fascicularis | BPRC | adult | 12 | Male and/or female | no | no |

Provide justifications for these choices

| | |
|---|---|
| Species | This project is a follow up of CCD licence [redacted]20185885 in which we used Macaca fascicularis. There are major differences in the expression of BBB transporters between rodent and human whereas the profile is nearly identical between macaques and humans. Together, this makes macaques a relevant species for this in vivo research. |
| Origin | All animals will be obtained from the in-house BPRC breeding colony. |
| Life stages | Adult. For consistency the animals should be matched for age and bodyweight or if significantly different, then distributed equally between groups |
| Number | 12 |
| Gender | It is not expected that there are gender-dependent effects, therefore males and/or females can be included |
| Genetic alterations | No |
| Strain | No |

**C. Accommodation and care**

Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?

x Yes

No > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices

**D. Pain and compromised animal welfare**

Will the animals experience pain during or after the procedures?

No

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

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

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

The compound will be injected IV under sedation and at specific timepoints the animals will be euthanised, under sedation

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

The animals will experience some stress due IM injection of the anaesthetic

Explain why these effects may emerge.

The animals are socially housed during this study and need to be temporarily separated for sedation

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

In order to limit the amount of stress, animals will be trained to cooperate for sedation procedures

**E. 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 F

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

Given our earlier data, no humane endpoints are expected. However, it cannot be fully excluded that the higher doses of the compound will have some effect. Animals will be monitored closely and if they reach score 3 (diarrhoea) or score 2 (other), the animals will be taken out of the study and treated in consultation with the veterinarian.

| | Score | Measurement data |
|---|---|---|
| **General impression** | 0 | Normal behaviour |
| | 1 | Slow reaction |
| | 2 | Listless appearance, e.g. does not immediately come for threats or food items, uncharacteristic behavior and too much lying down |
| | 3 | Apathic, limited reaction to stimuli or other animal(s) in the cage |
| **Diarrhoea** | 0 | Normal |
| | 1 | Soft |
| | 2 | Mush for more than 2 days |
| | 3 | Watery for more than 2 days |
| **Respiration** | 0 | Normal respiration rate |
| | 1 | Faster than normal but normal behaviour |
| | 2 | Faster than normal and less active behaviour |
| | 3 | Compromised breathing |
| **Neurological symptoms** | 0 | No symptoms, normal behaviour |
| | 1 | Slight tremors |
| | 2 | Incoordination |
| | 3 | Ataxic |

Indicate the likely incidence.

<1%

**F. Classification of severity of procedures**

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).

Sedation and subsequent administration of the compound IV: moderate
At the end, the animals will be euthanised under sedation moderate

Cumulative discomfort: moderate. Animals will be euthanised at the end of this study.

**G. 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 | Reliable or validated in vitro transcytosis assays are not yet available for the primate system. The partner is currently working on validation of the mouse system using carriers that are highly effective in vivo but have yet to find an in vitro/in vivo correlation. Hopefully these in vitro BBB systems will become available sometime in the future, but at this point it is difficult to predict when. There are currently no in vitro methods to further determine passage of the BBB and predict he kinetics of compound accumulation in the brain. *In* silico modelling is suitable for risk assessment and the determination of initial doses. However, sufficient information regarding the targets, physiology and interaction with the compounds is needed for reliable *in silico* modelling. These data are not available for the compounds studied in this project.<br>TXP1 antibody able to shuttle therapeutic payloads to the brain across BBB was identified using phage antibody selection method. TXP1 was subsequently characterized as potentially potent BBB transported in vitro. TXP1 is specific to human and monkey TfR1 and did not react to TfR1 from other tested species. The molecule passed developability criteria for a clinical candidate. Subsequently, TXP1 was tested for brain penetration in NHPs and showed to be very efficient in crossing BBB.<br><br>Further development required for clinical translation necessitates more detail analysis in vivo. We propose to conduct a series of experiments to define blood PK of TXP1. Critical parameters will be defined and used to model optimal dosing strategy for maximal brain exposure of the molecule. When used with a therapeutic payload that would translate to significantly improved drug efficiency.<br><br>Due to significant differences in receptor-mediated transport between distantly related species the studies have to be conducted in NHPs to serve as reliable for human translation. Quantitative proteomic studies have shown that there are major differences in the expression of BBB transporters between rodent and human, however the profile is nearly identical between primates. |
| Reduction | The experiments will be conducted using minimal number of animals that would guaranty data robustness. The experiments will follow a priority list starting with 2 timepoints with a subsequent timepoint added only if necessary |
| Refinement | All procedures will be performed under anaesthesia and animals will be trained to cooperate as much as possible with all procedures to minimize stress. Cameras will be used to allow constant observation of the animals in order to determine deviant behavior to allow early intervention when needed. If needed, additional nutrients will be provided. |

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

x No

Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

**H. Re-use**

Will animals be used that have already been used in other animal procedures ?

No > Continue with question I.

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

The cumulative discomfort is this proposal moderate

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

x No

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

**I. Repetition**

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

not applicable

**J. Location where the animals procedures are performed**

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

X No > Continue with question K.

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.

## End of experiment

**K. Destination of the animals**

Will the animals be killed during or after the procedures?

No > Provide information on the destination of the animals.

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

To quantify brain penetration of the molecule the brain needs to be extracted.

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.

x Yes > Will a method of killing be used for which specific requirements apply?

X No > Describe the method of killing.

The animals will be anaesthetised and subsequently euthanised with an IV injection of pentobarbital

Yes > Describe the method of killing that will be used and provide justifications for this choice.

| If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals. |
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