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 *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 1 — Type of animal procedure: Intravenous injection of antibodies for **blood 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 identify blood PK of the blood-brain barrier (BBB) shuttle antibody, TXP1 and if there is a need for repeated administration. The results are used to model and identify the most optimal dosing strategy for clinical translation. A series of experiments include single-dose escalation studies and, depending on single-dose blood PK and brain PK (see appendix 2), a two-dose study will be done to delivery on the objectives.

In general, animals will be iv injected with TXP1 and isotype control antibody (G12) at defined concentrations (ranging from e.g., 1.35-13.5mg/kg). To determine levels of TXP1 or G12 in the blood, bloodsamples will be collected from the animals at different timepoints (1 before injection and ranging from 1h to 28 days post injection).

Depending on the data from the single dose administration and the brain PK (see appendix 2), it is decided if a two-dose approach is needed in order to obtain maximal brain exposure and targeted receptor saturation. To investigate if repeated administrations are required to maintain sufficient target receptor saturation a second dose will be administered. This will also provide further information of potential effects of the first administration on the second dose. The amount of compound to be injected and the time of administration of the second dose depends on the outcome of the PK of the single dose injection. The timepoint of injection will depend on the timepoint when the amount of TXP1 in blood starts to decrease, which is expected between day 1-7.

Plasma will be prepared and analysed for antibody concentration by ELISA.

The use of comparator antibody (G12) with no target binding ability allows us to identify PK parameter that include blood half-life ($T_{1/2}$ - blood) for distribution and elimination phase, receptor saturation point, dose- and time-dependent clearance, dose-dependent plasma exposure (AUC - blood), dose-dependent median residence time (MRT).

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

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

The blood samples (1ml volume) will be collected from femoral vein or alternative suitable vein under sedation. The blood collection timepoint include, for example:

1. Single-dose blood PK: pre-dose, 1h, 3h, 6h, 1d, 3d, 7d, 14d, 21d and 28d
2. Second dose (Multi-dose) blood PK (example): pre-dose, 3h, 6h, 1d, 3d, 3d+3h, 3d+6h, 3d+1d, 3d+3d, 3d+7d, 3d+14d.

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 earlier results 2 animals per group are sufficient in this study to obtain reliable results.

A maximum number of 16 animals was predicted for the full blood PK study, with two animals per group. These include:

1. Single-dose escalation study – 12 animals - three concentrations with two compounds and two animals per group.
2. Multi-dose study – 4 animals – single concentration with two compounds and two animals per group.

Based on our previous experience from NHP and rodent PK studies we expect the variability level of <6 %CV. Consequently, our earlier studies justify the use of two animals per group per concentration per compound. The data generated from these experiments are expected to be of sufficiently low variability to generate significant information 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
1	Macaca fascicularis	BPRC	adult	16	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 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	16
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.

x Yes > 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 and subsequently blood samples will be collected. Therefore, all procedures will be performed under sedation.

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

The animals will experience some stress and lose weight due to repeated sedations.

Explain why these effects may emerge.

The animals are socially housed during this study and for sedation, the animals need to be temporarily separated to make the IM injection possible. Repeated sedations require repeated fasting procedures.

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 IM injection of the anaesthetic and get used to this procedure. In consultation with the veterinarian, the animals will be provided with additional nutrients e.g. by gavage or adapted food, to limit weight loss due to repeated sedations.

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 IV administration of the compound: light
Sedation for bloodsampling: light

Cumulative discomfort: moderate due to regular sedations

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	There are currently no in vitro methods to determine blood PK using the type of compounds studied in this project. *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. 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. 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. 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 with subsequent experiments 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. When needed, additional nutrients will be provided to the animals.

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 experiment should not have any lasting effects on the animals. The cumulative discomfort in this proposal is 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?

x No > Provide information on the destination of the animals.

After recovery of the last scheduled anaesthesia, the animals remain in their home cage. Due the cumulative discomfort level of moderate, they will be available for re-use in another study.

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

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

No > Describe the method of killing.

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.