



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 (0900-2800028).

1 General information

- 1.1 Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'. [REDACTED]
- 1.2 Provide the name of the licenced establishment. Biomedical Primate Research Centre
- 1.3 List the serial number and type of animal procedure
- | Serial number | Type of animal procedure |
|---------------|---|
| 1 | Safety and immunogenicity evaluation of a therapeutic vaccine for HER2 positive tumours |
- Use the numbers provided at 3.4.3 of the project proposal.*

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 direct aim of the experiments is to evaluate the VLP-HER2 vaccine for occurrence of adverse effects and immunogenicity in rhesus macaques. The VLP-HER2 vaccine to be evaluated aims at inducing antibody responses against the HER2 antigen and is developed for treating HER2 positive tumours in humans. Animals will be immunised with different VLP-HER2 vaccine formulations (i.e. varying doses with or without adjuvant) after which safety and immunogenicity will be monitored.

The primary outcome parameters of this project are:

Absence of unexpected reactogenicity of the vaccine: effects of the vaccine on general behaviour, health, body weight, local reactions and blood parameters.

Immunogenicity: Induction of cellular and humoral immune responses. The type and strength of the induced antibody response against the HER2 protein will determine if the objectives of the vaccine strategy are achieved and whether immune responses are sufficiently strong (i.e. similar to the Herceptin therapeutic concentrations ranging between 80- 115 µg/mL)¹ to proceed to clinical testing of the vaccine. The antibody levels induced by the vaccine will be compared to previously established therapeutic levels of HER2 monoclonal antibodies (mAbs).

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

Animals will receive one or more immunisations, typically at 4-to-8-week time intervals, although occasionally a longer time frame may be required between immunisations. Usually, 3 immunizations suffice over a period of 20 weeks (maximally 5 immunisations). However, when the longevity of immune responses and optional boosts need to be investigated, these limits may have to be exceeded. Specific rationale will then be presented to the animal welfare body (AWB). Immunisations will be done by intramuscular injection. Before and after each immunisation (maximally 4 times) blood will be collected for the monitoring of clinical chemistry and haematology parameters as well as for monitoring humoral and cellular immune responses. The maximum amount of blood taken will not exceed 1% of the body weight per month or 0.7% of the body weight per time point.

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

The number of animals will be based on statistical power analysis. Calculations take into account the number of animals needed to establish statistically significant differences of immune responses in relation to treatment group. Different antigen dosages with or without immunological adjuvant will be evaluated.

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	Rhesus macaque	Purpose bred	adult	60	female	Not applicable	Not applicable

Provide justifications for these choices

Species	The HER2 ectodomain of rhesus macaques is 99% homologous to the human HER2 protein ² . Therefore, a vaccine against a human self-antigen can be evaluated in rhesus macaques.
Origin	Purpose bred pedigreed animals from the Institute's colony will be used.
Life stages	Adult females will be used. The HER2 antigen is potentially expressed in breast tissue.
Number	The number of animals requested is based on the assumption that each study will have two vaccine groups (receiving different dosages or formulations) with 10 animals per group. In all, we anticipate performing 3 such studies over a 5-year period, the total number of animals needed will be maximally 60
Gender	Females will be used because of the presence of vaccine self-antigen in breast tissues
Genetic alterations	Not applicable
Strain	Not applicable

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?

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

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.

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

1. Discomfort due to repeated sedations
2. Discomfort due to injection of vaccine
3. Discomfort due to repeated blood sampling
4. Stress due to sedation and recovery

Explain why these effects may emerge.

1. Sedation can cause nausea and reduced appetite
2. When vaccines are administered intramuscularly this can cause pain and irritation
3. Repeated vena-punctures may cause pain and rarely haematoma's
4. Animals may experience disorientation when recovering from sedation

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

1. Sedation time will be kept as short as possible for the procedure, using the lowest dosage of sedatives required.
2. Animals will be sedated for vaccine delivery. Only rarely are strong adverse effects seen. Should granuloma formation be observed, the animal will be sedated, the wound will be cleaned, and analgesics are applied if necessary following veterinary consultation.
3. Vena punctures will be done by certified animal technicians and pressure is applied following collection to minimise the risk of haematoma.
4. Animals will be closely monitored, and a veterinarian will be consulted in case of problems.

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

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

Indicate the likely incidence.

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).

The total amount of discomfort is estimated as moderate. This is mainly caused by repeated sedations, immunisations and blood sampling.

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	The immune system is very complex and the <i>in vivo</i> interactions between virus and/or vaccine and host are not completely understood. At present there is no <i>in vitro</i> model available that can mimic the human immune system sufficiently to study the potential of a vaccine to induce immune responses to a self-antigen. The human and rhesus HER2 proteins have 99% sequence homology ² , which makes the rhesus macaque an appropriate model to establish the safety and immunogenicity of a therapeutic HER2 vaccine. The macaque immune systems closely resembles that of humans, facilitating translation to humans. The potential of this vaccine has previously been demonstrated in murine tumour models ³ .
Reduction	The number of animals needed per experiment will be based on statistical power calculation for achieving statistically significant differences in immune responses in the vaccine groups receiving different dosages or formulations. Only the minimum number of animals needed will be used.
Refinement	Animals are trained to cooperate as much as possible for the invasive handlings, such as receiving the sedation. Animals will be socially housed with a socially compatible animal, whenever possible. There is an extensive program for enrichment in our institute that consists of playing material and methods to present food (http://www.bprc.nl/en/welfare/). During the study animals will be observed daily by qualified and competent animal caretakers. Should changes occur in behaviour, appetite or stool then a veterinarian will be informed, and measures will be discussed with the investigator and implemented. Possible local reactions at the injection site of the vaccine will be recorded at multiple time points using a scoring system that includes redness, swelling and induration ⁴ . In case substantial induration is seen, then the wound will be treated, and analgesics will be applied. On every time point where a procedure is performed the animals will be weighed, body temperature measured, and closely examined. The animals to be used in the experiments will be selected on the highest HER2 antigen homology with the human HER2 antigen. This will be done using materials readily available from the BPRC DNA bank.

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

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 only requirement for the animals is that they are immune competent. Therefore, animals can be used that have been part of previous (infectious disease vaccine) studies.

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.

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

In most cases animals can be re-used if the total discomfort experienced allows. In some cases, it may be necessary to perform histopathology to exclude adverse events

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.

Euthanasia is done by injection of an anaesthetic dose of ketamine+medetomidine followed by an overdose of barbiturate intravenously

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.

References

- 1 Leyland-Jones, B. Dose scheduling--Herceptin. *Oncology* **61 Suppl 2**, 31-36, doi:10.1159/000055399 (2001).
- 2 Fattori, E. *et al.* ErbB2 genetic cancer vaccine in nonhuman primates: relevance of single nucleotide polymorphisms. *Hum Gene Ther* **20**, 253-265, doi:10.1089/hum.2008.153 (2009).
- 3 Palladini, A. *et al.* Virus-like particle display of HER2 induces potent anti-cancer responses. *Oncoimmunology* **7**, e1408749, doi:10.1080/2162402X.2017.1408749 (2018).
- 4 Draize, J. H., Woodward, G. & Calvery, H. O. Method for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J Pharmacol Exp Ther* **82**, 377-390 (1944).