Centrale Commissie Dierproeven

Bijlage
Beschrijving dierproeven

- Deze bijlage voegt u bij uw projectvoorstel dierproeven.
- Per type dierproef moet u deze bijlage invullen en toevoegen.
- Meer informatie vindt u op de website www.centralecommissiedierproeven.nl.
- Of neem telefonisch contact op. (0900-2800028).

1 Algemene gegevens

1.1	Vul uw deelnemernummer van de NVWA in.	50200
1.2	Vul de naam van de instelling of organisatie in.	BPRC
1.3	Vul het volgnummer en het type dierproef in. *Gebruik de volgnummers van vraag 3.4.4 van het format Projectvoorstel.*	Volgnummer: 1 Type dierproef: Evaluation of pharmacokinetics and immunological activity of MVA-IL7-Fc after a single intravenous administration in cynomolgus monkeys

2 Beschrijving dierproeven

A. Experimentele aanpak en primaire uitkomstparameters

Beschrijf de keuze van de experimentele aanpak en de primaire uitkomstparameters.

In the current proposal a novel therapeutic modality that aims to expand and improve the function of T cells and stimulate innate immune cells will be evaluated in cynomolgus monkeys as a mandatory step for further clinical development as a treatment for sepsis patients, with an impaired immune system, after surviving the acute phase of the disease. In the experimental design we selected we want to determine the pharmacokinetic profile and study the immunoregulatory effect of the compound in the absence of adverse events. Animals will receive a single dose of the therapeutic compound by intravenous administration. Venous blood will be collected from the groin before and at 4 timepoints after injection of MVA-hIL7-Fc in the first 24 hours, daily until day 7 and on day 14 (the last day) of the study (for PK analysis) for: a) pk-analysis of the compound (hIL7-Fc), b) study the immune activation of T cells and leucocyte subset numbers, c) hematology and d) clinical chemistry. The clinical chemistry and hematology analysis will serve to monitor unforeseen side effects, like changes in liver, kidney function etc. The study duration will be maximum 2 weeks after an intravenous injection of a single dose of the compound. There is only one experimental group receiving a single bolus injection of the compound MVA-hIL7-Fc.The dose selected for this experiment is the maximum expected dose MVA-hIL7-Fc to be produced under GMP conditions for clinical use, which was calculated on the basis of FDA Guidelines (“Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers”; “Table 1: Conversion of Animal Doses to Human Equivalent Doses Based on Body Surface Area”).

Criteria to select this compound for further testing:
• The test compound hIL-7-Fc has been shown to activate T cells in short term in vitro stimulation cultures with whole blood of cynomolgus monkeys.
• Preliminary data provided by the collaborator have shown beneficial effects of administration of MVA-hIL7-Fc in a rodent (murine) model of sepsis.
• The evaluation in non-human primates is part of the pre-clinical evaluation of the therapeutic agent and constitutes a go/no-go decision.

The primary outcome parameters are:
• Detectable blood-levels of hIL7-Fc in the 2-week follow-up after intravenous injection of MVA-hIL7-Fc to establish a pharmacokinetic profile.
• Establish immunoregulatory activity of hIL7-Fc by measuring T cell activation by pSTAT5 expression (hIL7-Fc binding to the IL7 receptor on T cells results in phosphorylation of STAT5 – pSTAT5 – signifying T cells activation) and T cell proliferation by expression of Ki67 (Ki67 is upregulated in proliferating T cells). In addition, we will analyse changes in leukocyte subset composition of immune cells in blood.
• The general immunoregulatory effect of injection of the compound on peripheral blood will be analysed by transcriptomics (Paxgene tubes)

The secondary outcome parameters are:
• Clinical behavioural symptoms as indicators of animal well-being.
• Clinical chemistry and haematology parameters will inform us on any unforeseen adverse events after administration of MVA-hIL7-Fc.

Beschrijf de beoogde behandeling van de dieren (inclusief de aard, de frequentie en de duur van de behandelingen waaraan de dieren worden blootgesteld) en onderbouw de gekozen aanpak.

The animals will be injected intravenously with a single bolus injection of the compound (injection time < 5 min). Venous blood will be collected from the groin before and at 4 timepoints after injection of MVA-hIL7-Fc in the first 24 hours, daily until day 7 and on day 14 (the last day). The blood collected at selected timepoint will be used for pharmacokinetic analysis of hIL7-Fc in serum, to establish the immunoregulatory potential of the compound and its effect on different leucocyte subsets by flowcytometry analysis, transcriptome analysis (Paxgene tubes) and hematology and clinical chemistry (as described above). In general, the maximum blood volume collected will not exceed 1% of the body weight per 2 weeks of this study (and 0.7% max per bleeding). Both injections of the compound and blood collection will be performed under sedation. During daily blood collection in the first week the animals will receive supplementary nutritious and calorie-rich diet by gavage to minimize the possible negative effects of fasting for the purpose of frequent sedation.
The study will be performed in a stacked manner. To forestall on unforeseen acute adverse responses, first 1 animal will receive a dose of the compound. Administration of the compound will be performed under sedation, by a veterinarian. Acute reactions will be observed by the veterinarian. Both heart rate and breathing rate will be monitored. After every blood collection the animals will be allowed to recover from the sedation and monitored by the animal caretaker/biotechnician on behavior, alertness and posture which will be recorded digitally in the clinical observation forms. In addition, camera surveillance will be installed during the 1st 24 hours after administration of the compound, for regular observation during the night. In consultation with the veterinarian we will decide to proceed with the remaining three animals.

Geef aan welke overwegingen en statistische methoden worden gebruikt om het aantal benodigde dieren tot een minimum te beperken.

In order to provide a reliable estimator for the production levels of hIL7-Fc (pharmacokinetics) 3 animals (N=3; see table in blue) would be the minimal number. Performing a ***Paired t-test*** (every animal is its own control; see table) comparing hIL7-Fc levels *before* and *after* administration of MVA-hIL7-Fc with a power of 0.8 (= 1-β) and significance (p-value = α) of 0.05 we would require an effect size of at least 3 x standard deviation (SD; d = the number of SD). Adding one extra animal (n=4; in green), with a power of 0.8 and p-value of 0.05 would reduce effect size to 2 x SD and in addition increase the reliability of the parameter estimates of hIL7-Fc production levels.

Paired t-test

α	0,05
1 - β (= Power)	0,80

d values for N

N =	1	2	3	4	5
2 to 9	-	9,958	3,097	2,080	1,662
11 to 20	0,937	0,888	0,846	0,810	0,778

B. De dieren

Benoem de diersoorten, herkomst, geschatte aantallen en levensstadia. Onderbouw deze keuzes.

Cynomolgus monkeys (adult; single sex), 4 animals. As this is a proof of concept study we will perform the study in a single sex group thereby minimizing the number of animals.
Cynomolgus monkeys are the animals of choice to evaluate cytokine based immunomodulatory therapies. The reason is that cynomolgus monkeys are phylogenetically closely related to humans to allow sufficient cross recognition of therapeutic agents that are specifically designed to bind human target molecules. The phylogenetically proximity of nonhuman primates to humans also allows us to use the vast array of immunological reagents, like antibodies, for the analysis of the effect of the MVA-hIL7-Fc treatment. Furthermore, the similarity in the cellular composition and functional characteristics of the immune system and physiological responses allows adequate evaluation of pharmacokinetics, expected therapeutic effects as well as potential adverse events. Especially, with regard to the response to product hIL7[1] and the linked constant region Fc region of an antibody nonhuman primates, including cynomolgus monkeys, are highly similar to the human immune system.[2] Cynomolgus monkeys are the species of choice in the current study for the effect of hIL7 is better detected in cynomolgus monkeys and secondly, the formal regulatory toxicity studies (not performed at this institute) planned after successful completion of the current study, will be performed in cynomolgus monkeys.

References

1. Magalhaes I, Vudattu NK, Ahmed RK, Kuhlmann-Berenzon S, Ngo Y, Sizemore DR, Wehlin L, Weichold F, Andersson J, Skeiky YA, et al. (2010). High content cellular immune profiling reveals differences between rhesus monkeys and men. Immunology 131, 128-140. 2010/05/15.
2. Hogarth PM, Anania JC and Wines BD. (2014). The FcgammaR of humans and non-human primates and their interaction with IgG: implications for induction of inflammation, resistance to infection and the use of therapeutic monoclonal antibodies. Curr Top Microbiol Immunol 382, 321-352. 2014/08/15.

C. Hergebruik

Is er hergebruik van dieren?

Nee, ga door met vraag D.

Ja > Geef aan op basis van welke overwegingen hergebruik in dit geval acceptabel wordt geacht.

Animals to be used in these experiments may have been used in other studies provided that they did not previously receive the viral vector MVA, an immunomodulatory compound (like IL7) or human antibody (responding to the Fc part of the compound). The current MVA-hIL7-Fc construct has been graded by the GMO office as being level "DM-I" and "ML-I". It is a non-replicative virus and after 6 months the system will be cleared from the virus. The expression of hIL7-Fc is transitory. However, the discomfort classification of moderate, might interfere with the reuse of those animals. I would not recommend to reuse these animals for an experiment in which MVA is involved. Given the long lifespan of this species reuse will take place in the legal framework described in art. 1 of the law on animal testing.

Is er in het voorgaande of in het geplande gebruik sprake van (of een risico van) ernstig ongerief?

Nee

Ja > Geef aan op basis van welke overwegingen hergebruik in dit geval acceptabel wordt geacht.

nvt

D. Vervanging, vermindering en verfijning

Laat zien hoe de toepassing van methoden voor vervanging, vermindering en verfijning zijn meegewogen bij het bepalen van de experimentele strategie, de keuze van de dieren en de opzet van de dierproef en welk keuzes daarbij zijn gemaakt.

Replacement: We have first tested *in vitro* that the compound mediates the expected immunoregulatory effect on blood cells by flowcytometry. In mice, the equivalent dose of MVA-IL7-Fc injected in this experiment was found to be safe and effective in a mouse model of sepsis after treatment with MVA-hIL7-Fc as compared to mice treated with MVA alone.
However, further in vivo studies are needed for new drugs (i.c. MVA-hIL7-Fc), especially biopharmaceutical compounds, that have an effect on the immune system in a relevant non-rodent species before it can be used in human. This species has to be sensitive for the intended action of the therapy as well as the possible side-effects. In this way we can obtain relevant information on safety after intravenous administration of this compound, actual serum levels of hIL7-Fc that will be produced after administration of MVA-hIL7-Fc and what the effect of MVA-IL7-Fc on the immune system will be. The immune system in nonhuman primates display a similar complexity as the human immune system, is well characterized and has the required immunological tools for a good analysis of the immunological activity of MVA-IL7-Fc. Currently, there are no alternatives *in vitro* or *in silico* for pk studies of these new compounds.
Reduction: The study will be performed in a stacked manner. To forestall on unforeseen acute adverse responses, first 1 animal will receive a dose of the compound This animal will be monitored for any acute adverse reaction to the administration of the MVA-hIL7-Fc for 24 hrs. In consultation with the veterinarian we will decide to proceed with the remaining three animals. Only the minimum number of animals needed will be used. As this is a proof of concept study the study will be performed in a single sex thereby minimizing the number of animals. To obtain relevant pharmacokinetics and allow for possible variation of the effect of the compound on the immune system the study will be carried out in 4 animals.
Refinement: Supplementary nutritious and calorie-rich diet is administered (via gavage) when animals are sedated daily for blood sampling. The gavage is given during the sedation for a period of seven days. This reduces the possible negative effects of fasting for the purpose of frequent sedation. The number of blood samplings, and the collected volumes of blood are reduced to a minimum. All observations will be documented and registered in a clinical scoring form which is part of the digital database at the institute. In the context of this pharmacokinetic study we would like to introduce the remote monitoring of the behaviour of the animals, in the first 24 hrs and on indication of abnormal behaviour, by placing video cameras in the stable.

Geef aan welke maatregelen zijn genomen om de kans op pijn, lijden of angst bij de dieren en de kans op nadelige milieueffecten tot een minimum te beperken.

To minimize the discomfort to the animals they will be sedated to facilitate animal handling and performance of the technical procedures. Each time an animal is sedated, the animal will be weighed, the body temperature will be taken, and the animal will be closely examined. Our institute uses a customized database that documents all individual animals in the institute. General observations like behaviour, appetite and stool are part of this database. This database therefore facilitates early recognition of minor changes in these general parameters. The procedures will be performed on fully sedated animals and pain is not anticipated. Regular analysis of haematological and clinical chemistry parameters is part of the experiment. If necessary, judged by the veterinarian, measures will then be taken to treat the animal. The studies will be performed according the guidelines of the “Wet Milieubeheer”, and will cause no adverse effects on the environment.

Herhaling en duplicering

E. Herhaling

Geef aan hoe is nagegaan of deze dierproeven niet al eerder zijn uitgevoerd. Indien van toepassing geef aan waarom duplicatie noodzakelijk is.

n.v.t.

Huisvesting en verzorging

F. Huisvesting en verzorging

Worden de dieren anders dan volgens de eisen in bijlage III van de richtlijn 2010/63/EU gehuisvest en/of verzorgd?

Nee

Ja > Geef, indien dit kan resulteren in nadelige effecten op het dierenwelzijn, aan op welke wijze de dieren worden gehuisvest en verzorgd en motiveer de keuze om af te wijken van de eisen in bovengenoemde bijlage III.

G. Plaats waar de dieren worden gehuisvest

Worden de dierproeven geheel of gedeeltelijk uitgevoerd bij een inrichting die niet onder de rechtstreekse verantwoordelijkheid van een instellingsvergunninghouder Wod valt?

Nee > Ga verder met vraag H.
NEE

Ja > Geef aan wat voor bedrijf of instelling dit betreft.

Waarom is hiervoor gekozen en hoe wordt een adequate huisvesting, verzorging en behandeling van de dieren gewaarborgd?

Ongeriefinschatting/humane eindpunten

H. Pijn en pijnbestrijding

Valt te voorzien dat er pijn kan optreden bij de dieren?

Nee > Ga verder met vraag I.

Ja > Worden in dat geval verdoving, pijnstilling en/of andere pijnverlichtingsmethoden toegepast?

Nee > Motiveer dan waarom geen pijnverlichtingsmethoden worden toegepast.

Ja > Geef dan aan welke pijnverlichtingsmethoden worden toegepast en op welke wijze wordt verzekerd dat dit op een optimale wijze gebeurt.

The procedures will be performed on fully sedated animals and pain is not anticipated.

I. Overige aantasting van het welzijn en maatregelen

Welke eventuele andere vormen van welzijnsaantasting worden voorzien?

1. Response to the injection of the MVA-hIL7-Fc. Although only minimal reaction have been described for both individual components there might be an unanticipated response.
2. Discomfort as a consequence of the sedation

Geef aan wat de mogelijke oorzaken hiervan zijn.

Ad1) Although minimal adverse reactions have been reported for each individual component MVA or IL-7 in both human and nonhuman primates and while no adverse reaction were observed in mice for MVA-hIL7-Fc we will stay alert for unanticipated effects.
Ad2) Disorientation and nausea when recovering from the sedation.

Beschrijf welke maatregelen worden genomen om deze schadelijke effecten te voorkomen of waar mogelijk te minimaliseren.

Ad 1) Animals will be monitored closely by the veterinarian during and after administration of MVA-hIL7-Fc to act quickly on unforeseen events.
Ad 2) Although disorientation cannot be prevented, animals will be fasted overnight before sedation to prevent vomiting.

J. Humane eindpunten

Valt te voorzien dat zich bij deze dierproef omstandigheden voordoen waarbij het toepassen van humane eindpunten geïndiceerd is om verder lijden van de dieren te voorkomen?

Nee > Ga verder met vraag K.

Ja > Geef aan welke criteria hierbij worden gehanteerd.

Welk percentage van de dieren loopt kans deze criteria te halen?

Based on the type of experiment a human endpoint is not expected. Though when severe adverse symptoms are observed, directly the veterinarian will be consulted and appropriate action will be taken. Based on experience, we anticipate a drop out of <1%.

K. Classificatie van ongerief

Geef aan hoe in het licht van alle hierboven beschreven negatieve effecten het cumulatief ongerief wordt geclassificeerd in termen van *'terminaal', 'licht', 'matig'* of *'ernstig'* ongerief.

Sedation, injection with MVA-IL7-Fc and blood collection in the context of this pharmacokinetic study are considered mild levels of discomfort. However, the frequency of blood sampling/sedation in the first 24 hrs is considered moderate discomfort. So overall, the level of discomfort will be classified as moderate.

Einde experiment

L. Wijze van doden

Worden de dieren als onderdeel van het experiment of na afloop van het experiment gedood?

Nee

Ja > Geef aan waarom het doden van dieren als eindpunt essentieel is voor deze proef.

Wordt er een methode(n) van doden uit bijlage IV van richtlijn 2010/63/EU toegepast?

Nee > Beschrijf de euthanasiemethode en onderbouw de keuze hiervoor.

Ja