Centrale Commissie Dierproeven

Dit is een kopie van het CCD formulier, waarbij de invulvelden niet beveiligd zijn. Voor indiening bij de CCD moet de door de DEC goedgekeurde versie in het CCD formulier worden overgezet.

Versie CCD formulier dd. 2016-03-02

Format

Projectvoorstel dierproeven

- Dit format gebruikt u om uw projectvoorstel van de dierproeven te schrijven
- Bij dit format hoort de bijlage Beschrijving dierproeven. Per type dierproef moet u deze bijlage toevoegen.
- Meer informatie over het projectvoorstel 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.	Biomedical Primate Research Centre

1.3 Vul de titel van het project in.

Evaluation of pharmacokinetics and immunological activity of MVA-IL7-Fc after a single intravenous administration in cynomolgus monkeys

2 Categorie van het project

2.1 In welke categorie valt het project.

U kunt meerdere mogelijkheden kiezen.

De verplichte bijlagen verschillen per categorie.

Op hetInvloket.nl leest u meer informatie over de verplichte bijlagen per categorie.

Fundamenteel onderzoek
X 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 Algemene projectbeschrijving

3.1 Achtergrond

Licht het project toe. Beschrijf de aanleiding, de achtergrond en de context. Besteed aandacht aan de bij vraag 2 aangekruiste categorieën.

- Geef in geval van 'wettelijk vereiste dierproeven' aan welke wettelijke eisen (in relatie tot beoogd gebruik en markttoelating) van toepassing zijn.
- Geef in geval van 'routinematige productie' aan welk(e) product(en) het betreft en voor welke toepassing(en).
- Geef in geval van 'hoger onderwijs of opleiding' aan waarom in dit project, in relatie tot het opleidingsprogramma en eindtermen, is gekozen voor dierproeven.

Introduction: Different diseases can cause severe suppression of the immune system, either caused by the disease or as a consequence of the treatment. Even after effective treatment many of these patients are confronted with an impaired immune system for a long period of time. Infectious diseases like sepsis and aids and certain forms of cancer are examples of diseases causing severe suppression of the immune system. During HIV infection, important regulator cells of the immune system are destroyed [1]. After the acute phase of sepsis, the majority of patients develop immune suppression[2]. In cancer patients, tumours can suppress the immune system in order to avoid detection and destruction [3]. After effective treatment of the infection or cancer, the immune system should restore to its normal function. However, even if the infection is under control or the cancer is cured, in many patients the immune system stays impaired, leaving them vulnerable to new infections and/or cancer. The MVA-hIL7-Fc compound that is evaluated in the current study is developed for a clinical of established immune-depression characterizing a majority of sepsis patients who survive the acute phase of the disease. The treatment with MVA-hIL7-Fc aims to restore immune function of both the innate and adaptive system after established immune-depression in sepsis patients.[2] In particular, lymphopenia has been linked to poor clinical outcome. We anticipate and are aiming for a limited number of injections of the MVA-products (perhaps 2) in the clinic. Patients targeted in the clinic typically will stay in ICU for 2-3 weeks at most. So, a single injection needs to demonstrate a good activity (as we have seen in murine models at an equivalent dose) in this timeframe and this is what is expected to be achieved in the proposed study. A Protocol for First-In-Man study (phase 1) is currently being drafted, based on the assumption that the current study in NHP will demonstrate adequate pharmacokinetics and activity of the product.

Restoring Immune function with cytokine therapy: The novel immunomodulating compound evaluated in the current proposal aims to achieve restoration of immune function by incorporating 3 components. **The first component** constitutes the cytokine interleukin 7 (IL7). IL7 is a non-hematopoietic cell-derived cytokine that mediates adaptive immune homeostasis by enhancing the proliferation and function of T cells[4]. Binding of IL7 to the IL7 receptor on T cells, results in intracellular signalling leading to phosphorylation of signal transducer and activator of transcription 5 (STAT5), mediating proliferation and improved functionality of those T cells.[5] Importantly, the IL7 response network contains an intrinsic regulatory feedback resulting in downregulation of IL7 mediated responses in case of overproduction of IL7 [4]. This security net prevents overstimulation of the immune system. When MVA-hIL7-Fc is injected in healthy immunocompetent mice (hIL7 is active in mice since it is cross-reactive with the murine IL7 receptor – CD127), the safety biological feedback is observed by a decrease in the number and percentage of T cells expressing CD127 as well as a decrease in the overall expression of the CD127 for a few days after injection. Then T cell numbers and proportions of CD127 expressing T cells and overall expression of CD127 returns to normal levels. In conclusion, the hIL7-Fc does not alter the safety biological feedback of IL7 and we did not observe any abnormal effects. The expected biological activities of the product were observed, as demonstrated by the induction of some immune cells due to MVA and increase in the overall T cell numbers due to hIL7. Therapy

with human IL7 (hIL7) in cynomolgus monkeys has been shown to alter peripheral homeostasis in both T-cell-repleted (healthy) and T-cell-depleted (SIV infected) nonhuman primates. [6] Treatment with hIL7 of lymphopenic HIV-infected patients induced a sustained increase of naive and central memory CD4+ and CD8+ T cells. [7] Treating cancer patients with incurable malignancies with IL7, showed marked increase in peripheral CD3+, CD4+, and CD8+ lymphocytes.[8] Treatment with IL7 was well tolerated in both nonhuman primates and human and resulted in increased T cell counts and improved functionality of the immune system.

The compound we aim to evaluate in the current proposal contains the hIL7 gene fused to **the second component** which consists of the constant region of the human IgG2a antibody the so-called Fc domain (Fc = crystallizable fragment). This Fc domain mediated binding to the so-called neonatal Fc receptor (FcRn) rescuing proteins linked to it from rapid degradation by recycling the fused protein, thereby increasing its half-life and persistent action. [9]

The third component is the viral modified vaccinia Ankara (MVA) vector for the delivery of the hIL7-Fc gene. MVA infects both immune and non-immune cells instructing those cells to produce the protein included in the MVA, in this case the hIL7-Fc. The infected cells will provide a prolonged production of hIL7-Fc protein and as a consequence a prolonged activity of hIL7-Fc. MVA has the added advantage that it contributes to the immune potentiating effect by stimulating the innate immune system. The replication-deficient MVA was used to vaccinate over 100.000 people against smallpox with an excellent safety record [10]. MVA is also widely used as a delivery vector for vaccines against infectious disease. MVA, carrying TB antigens, have been evaluated as vaccines against TB in nonhuman primates by our group[11, 12]and in human by others.[13]

In summary, the current compound MVA-hIL7-Fc aims to restore function in both the innate and adaptive immune system by combining for the first time a cytokine (hIL7) enhancing both proliferation and function of T cells, linked to an Fc tail prolonging its action, with a delivery platform (MVA) displaying the capacity to stimulate the innate immune system and prolonged production of the hIL7-Fc fusion protein.

A final dimension to the program is the use of the intravenous (iv) route for administration of the MVA-hIL7-Fc. In a recent study, intravenous administration of MVA, in human, was found to be safe when it was used as a vaccine vector carrying malaria proteins.[14]

Aim of the study: In this exploratory proof of concept study we want evaluate pharmacokinetics of MVA-hIL7-Fc in cynomolgus monkeys and establish the immunological activity of MVA-hIL7-Fc after intravenous injection of a single dose. IL-7 is required for T cell development and for maintaining and restoring homeostasis of mature T cells. IL-7 is a limiting resource under ***normal*** conditions, but it accumulates during lymphopoiesis, leading to increased T cell proliferation. The administration of recombinant human IL-7 to ***normal/naïve*** non-human primates and humans has been shown to result in widespread T cell proliferation, increased T cell numbers, modulation of peripheral T cell subsets and increased T cell receptor repertoire diversity.[4, 15] As such healthy immunocompetent animals will provide a relevant model to evaluate both the pharmacokinetic parameter as its immunoregulatory potential that we analyze by flowcytometry. In addition, data generated in naïve/normal mice and sepsis-CLP mice, have shown comparable levels of MVA-produced hIL7-Fc. It is important to establish that, after intravenous administration with the highest expected dose of MVA-hIL7-Fc in an animal species closest to humans, we can show safety and that it regulates the immune-pathways it is expected to. The dose to be used in 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 [16]. Preliminary data in mice provided by the collaborator (personal communication) show that administration of MVA-hIL7-Fc through different routes (intravenous, intramuscular and subcutaneous) is safe in healthy mice with the iv-route providing the most optimal pharmacokinetics. The Cecal Ligation and Puncture (CLP) model of sepsis, that was used is for the evaluation of MVA-hIL7-Fc, is a major reference mouse sepsis model.[17-20] The activities of MVA-hIL7-Fc have been characterized for the treatment of the immune-suppressed status after the induction of sepsis, resulting in restoration of immune functions but also increase survival post-CLP.

Why in nonhuman primates: New treatments, especially biopharmaceutical compounds, that have an effect on the immune system need to be tested 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. The close evolutionary proximity of nonhuman primates, including cynomolgus monkeys, translates to a similar immunological complexity and regulation, making this species a highly relevant model for the evaluation of new therapies targeting the immune system. Especially with regard to components included in the current compound, the hIL7 and the linked Fc-protein, nonhuman primate models are of particular relevance. Nonhuman primates have a highly similar Fc-receptor repertoire and function[21] compared to humans and show comparable effects on STAT5 phosphorylation in response to stimulation of CD4+ and CD8+ T cells with IL-7,[22] the functional readout for immunological activity we are determining in this study. In addition, the phylogenetically proximity of nonhuman primates to humans allows us to use the vast array of immunological reagents, like antibodies, that also bind to analogous targets on cells of the nonhuman primate immune system required, for the analysis of the effect of the MVA-hIL7-Fc treatment. Cynomolgus monkeys are the species of choice in the current study for the effect of hIL7 is better detected in cynomolgus monkeys than in rhesus 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.

The information obtained in the experiment will provide us with sufficient information for further development for clinical use. The current protocol is a scientific, exploratory, proof-of-concept study that does not aim at replacing a Regulatory Study also to be performed in NHP. The generated data from the NHP study is necessary and will instill confidence on the use of the expected highest dose of MVA-hIL7-Fc in human.

3.2 Doel

Beschrijf de algemene doelstelling en haalbaarheid van het project.

- In het geval het project gericht is op één of meer onderzoeksdoelen: op welke vra(a)g(en) dient dit project antwoord(en) te verschaffen?
- In geval het een ander dan een onderzoeksdoel betreft: in welke concrete behoefte voorziet dit project?

The ultimate goal of this project is to obtain a new therapeutic compound stimulating both the adaptive and innate part of the immune system thereby restoring immune function in patients who, as a consequence of infection or treatment, had an impaired immune system.
The direct aim of the current study is to determine the pharmacokinetics of hIL7-Fc after intravenous injection of a single dose of MVA-hIL7-Fc with a follow-up of 2 weeks after injection. In addition, we want to analyse the biological effect of the hIL7-Fc production on the proliferation of T cells and other immune cell types in the blood and determine the effect on the functionality of T cells.
Our institute has extensive and long-standing expertise in conducting studies using nonhuman primates.[23] Different immunomodulatory therapies have been evaluated in the context of organ transplantation [24] and autoimmune diseases.[25] There is also extensive experience with PK studies that were performed in nonhuman primates in the context of these therapy evaluation studies. [26, 27]. The investigators involved have extensive experience with analysis of immune responses, immune-cell phenotyping with particular knowledge on T cell responses[12] analysis of cytokine responses and monitoring of well-being of the animals, including clinical chemistry and haematology evaluation.

3.3 Belang

Beschrijf het wetenschappelijk en/of maatschappelijk belang van de hierboven beschreven doelstelling(en).

Scientific/Translational impact: Treatment with IL7 have been found effective and safe in different patient groups. However, the new mode of delivery (IL7-Fc fusion gene with MVA) which adds to the therapeutic effect of the compound (MVA-IL7-Fc) and providing prolonged production of IL7-Fc can open up a new therapeutic strategy in delivering other immunomodulatory compounds with a short half-life. The current study will provide information on the levels of hIL7-Fc after administration of MVA-IL7-Fc and on the immunological activity of the MVA-IL7-Fc compound, that will inform further clinical development of this compound for the treatment of patients with an impaired immune system.

Societal impact: Different diseases, like infectious diseases and cancer can cause severe immune suppression, either caused by the disease or as a consequence of the treatment. Moreover, even after effective treatment, many of these patients are confronted with an impaired immune system for a long period of time. Resulting in increased susceptibility for common infections and a greater chance of recurring cancer. For this reason, these patients would benefit from strategies/therapies restoring immune function. MVA-IL7-Fc evaluated in the current proposal, has the potential to restore immune function of both the adaptive and innate immune system.

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3.4 Onderzoeksstrategie

3.4.1 Geef een overzicht van de algemene opzet van het project (strategie).

In this project proposal a novel strategy to restore immune function by expanding and enhancing the function of T cells (by hIL7-Fc) and stimulate innate immunity (by MVA vector) *in vivo,* will be evaluated in nonhuman primates as part of the pre-clinical development of MVA-hIL7-Fc. Preliminary data of MVA-hIL7-Fc has shown:

- The compound hIL7-Fc is immunologically active and has been shown to activate T cells in short term *in vitro* stimulation cultures of cynomolgus monkey blood cells resulting in phosphorylation of STAT5 (pSTAT5). In addition, we will measure the expression of the Ki67 marker in T cells, which is associated with proliferation. Expression of both markers constitute functional activity of the expressed hIL7-Fc.
- The compound that is being developed, MVA-hIL7-Fc, has been shown to mediate beneficial immunomodulatory effects in mice with no adverse effects.
- The evaluation of MVA-hIL7-Fc in nonhuman primates is part of its pre-clinical development.

Animals will receive a single intravenous injection with MVA-hIL7-Fc. Blood will be collected before and at selected timepoints over a 2-week period after injection to assess hIL7-Fc levels, changes in immune cell activity, number and phenotype and clinical chemistry and hematology parameters. Based on the calculation of the human equivalent dose (HED) used in the mouse experiments (3.3 x 10^6, 3.3 x 10^7 and 3.3 x 10^8 pfu MVA-IL7-Fc/kg respectively) and the calculated HED selected for the current experiment (5 x 10^7 pfu MVA-IL7-Fc/kg for cynomolgus monkeys) a 2-week follow-up period should provide sufficient time to obtain relevant pharmacokinetic data in cynomolgus monkeys and establish immunological activity. In the experiments performed in mice, the peak hIL7-Fc concentration in blood was detected between 6 and 24h post-injection of MVA-hIL7-Fc and still detectable up to 96 hours post-injection for the highest MVA-hIL7-Fc dose. Pharmacokinetics between mice and monkeys might differ. An ELISA will be performed after day 7 to determine the hIL7-Fc levels. 1) If no detectable levels of hIL7-Fc (< 1ng/mL) are found at day 7 or earlier, a follow-up period of 14 days will suffice to provide for a good estimation of the hIL7-Fc production. 2) If detectable levels of hIL7-Fc (< 1ng/mL) are still found at day 7 the follow-up period will be extended with an extra 7 days with an extra blood collection, for analysis, on the final day 21."

In addition, the immune potentiating effects related to the MVA-expressed hIL7-Fc were detected for the 3 tested doses in mice and detectable 2 weeks after the injection.

The primary study goal is to obtain information on the pharmacokinetics of hIL7-Fc and establish its immunoregulatory potential by measuring the expansion and activation of T cells in the absence of adverse events.

3.4.2 Geef een overzicht op hoofdlijnen van de verschillende onderdelen van het project en de daarbij gebruikte type(n) dierproef of dierproeven.

This proof of concept study consists of one type of experiment, namely the evaluation of MVA-hIL7-Fc in a pharmacokinetic study as described under 3.4.1.

3.4.3 Beschrijf en benoem de logische samenhang van deze verschillende onderdelen en de eventuele fasering in de uitvoering. Vermeld eventuele mijlpalen en keuzemomenten.

The proposal describes only one type of experiment. In this application we use a pharmacokinetic approach determining the levels of hIL7-Fc after intravenous injection of the MVA-IL7-Fc construct and its biological effect on the immune system.

This experiment has been formulated as a pivotal prerequisite for the further development of the compound: When failing to demonstrate that the product is active, the MVA-hIL7-Fc will not be further developed.

3.4.4 Benoem de typen dierproeven. Vul per type dierproef een bijlage Beschrijving dierproeven in.

Volgnummer	Type dierproef
1	Evaluation of pharmacokinetics and immunological activity of MVA-IL7-Fc after a single intravenous administration in cynomolgus monkeys
2	
3	
4	
5	