Centrale Commissie Dierproeven

Form
Project proposal

- This form should be used to write the project proposal for animal procedures.
- The appendix 'description animal procedures' is an appendix to this form. For each type of animal procedure, a separate appendix 'description animal procedures' should be enclosed.
- For more information on the project proposal, see 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'.	50200
1.2	Provide the name of the licenced establishment.	Biomedical Primate research Centre
1.3	Provide the title of the project.	Pre-clinical PK/PD evaluation in non-human primates of immuno-therapeutics aimed to induce regulatory T-cells.

2 Categories

2.1 Please tick each of the following boxes that applies to your project.

- ☐ Basic research
- ☒ Translational or applied research
- ☐ Regulatory use or routine production
- ☐ Research into environmental protection in the interest of human or
- ☐ Research aimed at preserving the species subjected to procedures
- ☐ Higher education or training
- ☐ Forensic enquiries
- ☐ Maintenance of colonies of genetically altered animals not used in other animal procedures

3 General description of the project

3.1 Background

Describe the project (motivation, background and context) with respect to the categories selected in 2.

- For legally required animal procedures, indicate which statutory or regulatory requirements apply (with respect to the intended use and market authorisation).
- For routine production, describe what will be produced and for which uses.
- For higher education or training, explain why this project is part of the educational program and describe the learning targets.

of the primary features of the immune system is its capacity to discriminate between self and non-self molecules. However, inadvertent reactions against self-molecules occurs in some cases and can lead to auto-immune diseases, such as type I diabetes, multiple sclerosis (MS), rheumatoid arthritis (RA),

myasthenia gravis (MG), systemic lupus erythematosus (SLE) etc. Furthermore, the immune reaction against non-self molecules will lead to rejection of foreign tissue in organ transplantation. In all these cases immunosuppressive treatment is indicated. Although immunosuppressive drugs are widely used to prevent transplant rejection and for the treatment of autoimmune disease, these drugs typically have a broad immunosuppressive effect leading to serious side effects, such as enhanced susceptibility to infectious disease, enhanced risk for malignancies or unwanted effects on non-immune cells, resulting in toxicity.

Regulatory T-cells in the treatment of disease. Regulatory T-cells (T_{REG}) are essential for suppressing inflammation and for the regulation of immune system activity (1, 2). T_{REG} are characterized by constitutive expression of the transcription factor FOXP3. The critical role of T_{REG} in the development of autoimmunity has been highlighted by the multi-organ autoinflammatory syndrome that develops in FOXP3 deficient mice (3, 4) and the immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome seen in humans which harbor mutations in FOXP3 (5, 6). T_{REG} are dependent on the cytokine IL-2 for their survival, proliferation and immune-suppressive function (2).

Many autoimmune and chronic inflammatory disorders, such as systemic lupus erythematosus (SLE), type 1 diabetes (T1D), chronic graft-versus-host disease (cGVHD), multiple sclerosis (MS) and rheumatoid arthritis (RA) feature numerical and/or functional deficiencies in T_{REG} cells associated with defective IL-2 production (7). Along these lines, stimulation and expansion of T_{REG} cells in autoimmune and chronic inflammatory patients has been tested using low dose-IL-2 treatment in SLE (8), steroid-refractory cGVHD (9), hepatitis C-induced cryoglobulinaemic vasculitis (10), and T1D (11). A recent study has further examined low dose-IL-2 treatment across 11 different autoimmune/inflammatory diseases and reported T_{REG} cell-activation in all treated individuals (12).

Although the low-dose IL-2 was well-tolerated and caused T_{REG} cell expansion, the therapeutic use of low-dose IL-2 is hampered by the following limitations: (i) IL-2 is very short-lived *in vivo* (half-life around 7-10 minutes) and requires daily injections; (ii) IL-2 has a very narrow therapeutic window since IL-2 is not selective per se and stimulates both T_{REG} as well as effector lymphocytes, potentially worsening immunopathologies; (iii) higher doses of IL-2 can cause toxic adverse effects leading to pulmonary edema and liver cell damage In addition, IL-2 can induce cytokine release and vascular leakage syndrome (see below) (1, 2, 13).

These limitations have led to the design of therapeutic mAbs that **bind to IL-2. To this end, specifc mAbs were developed that, combined with recombinant human IL-2, forms T_{REG}-biased IL-2/anti-IL-2 mAb complexes (briefly IL-2 complexes).** As such, these IL-2 complexes resulted in an altered capacity to bind to T_{REG} versus T immune-effector cells for more potent and selective T_{REG} expansion with longer half-life, reducing the frequency of injections significantly. The IL-2 complexes are selective for the trimeric IL-2 receptor (IL-2R) that is constitutively expressed by T_{REG} cells. The trimeric IL-2R consists of IL-2R alpha (CD25), IL-2R beta (CD122) and the common gamma chain (yc). The selectivity of T_{REG} cell-biased IL-2 complexes is based on the new anti-human IL-2 mAb that binds to very specific sites of IL-2, thus temporarily interfering with the interaction of IL-2 with CD122/CD132. The net result of such interaction is that interference with CD122/CD132 results in the selective and efficient delivery of IL-2 to immune cells expressing high levels of CD25, in particular T_{REG} cells. Injection of IL-2/anti-mouse-IL2 mAb complexes in mice showed that a selective and preferential stimulation of T_{REG} cells over cytotoxic CD8$^+$ T and NK cells is induced. (1, 14). These complexes did not show any toxic effects in the mice (15, 16). However, further evaluation of the humanized mAb version is needed in a suitable animal species before it can be tested in human clinical trials.

Evaluation in animal species. MAb that interact with the immune system can induce adverse immune-mediated drug reactions such as infusion reactions, cytokine release syndrome, anaphylaxis, immune-complex-mediated pathology and autoimmunity. Infusion of mAb can result in an anaphylactic shock if IgE responses are triggered or immune-complex deposition occurs. These reactions depend on the presence of pre-existing antibodies against the therapeutic mAb and only rarely occur upon first infusion, but can form a problem after prolonged use. However, an immediate strong release of inflammatory cytokines can be induced by unexpected interactions of the therapeutic mAb with immune-effector cells or via triggering of innate immune cells, such as natural killer (NK) cells and phagocytic cells, or complement, through Fc Receptor (FcR) interactions (17). The ensuing cytokine release syndrome can lead to systemic organ failure and result in death, as observed in a phase I clinical trial where the CD28 superagonist mAb TGN1412, targeted for use against RA, was tested (18). Cytokine release syndromes have also been recorded in mAb therapies targeting CD3, expressed on T-cells, and during IL-2 cytokine therapy (1).

The potential risks associated with mAb immune-therapy in general and T_{REG} inducing mAb therapy in particular, has implications for the pre-clinical evaluation of these therapies. Special emphasis has to be put on the effect on the activation and proliferation as well as function of different immune cell populations. In addition, potential triggering of a cytokine release syndrome has to be considered. This requires testing in suitable *in vitro* and *in vivo* animal models (17). Extensive *in vitro* studies involving immunopharmacology on the appropriate cell types and tissue cross-reactivity studies need to be followed by studies in

appropriate animal species. Differences in target molecule sequence homology, expression pattern and function as well as the use of human or humanized mAb precludes the use of many rodent species and makes it necessary to use non-human primates, such as macaque species, that are more closely related to humans. In addition, the much closer similarity in FcγR expression and innate effector function, which are essential to study FcR mediated adverse events associated with the use of mAb therapy, and homology in immunoglobulin genes (important for studying induction of Ab responses to the therapeutic mAb) makes the use of non-human primates pivotal (19-23). While other species can be used to obtain proof of principle by testing surrogate mAb binding to a homologous target, non-human primates are considered the only relevant species for dose range-finding (DRF) and pharmacokinetic/pharmacodynamic (PK/PD) evaluation before clinical trials can start (17).

3.2 Purpose

Describe the project's main objective and explain why this objective is achievable.

- If the project is focussed on one or more research objectives, which research questions should be addressed during this project?
- If the main objective is not a research objective, which specific need(s) does this project respond to?

The ultimate goal of this project proposal is to obtain a new therapeutic method, based on the *in vivo* amplification and activation of regulatory T-cells, that can be used for treatment of autoimmune diseases, such as RA, ankylosing spondylitis, psoriasis, MS, SLE, and against organ rejection in transplantation.
The direct aim is to evaluate a newly developed humanised anti-human IL-2 monoclonal antibody (mAb) in combination with recombinant human IL-2, forming T_{REG}-biased IL-2/anti-IL-2 mAb complexes (briefly IL-2 complexes, for their potential to selectively activate and increase the number of T_{REG} in non-human primates and to monitor for unexpected adverse events, such as cytokine release syndrome. Currently, a phase 2 clinical trial with low-dose IL-2 is ongoing in SLE patients. The IL-2-mediated changes in the immune system and disease activity observed in the SLE patients will serve to guide decisions on the suitability of these patients for IL-2 complex therapy.

In addition to SLE, the principle of IL-2 complex-based T_{REG} cell expansion could be extended to diseases currently treated with immunosuppressive drugs, such as other above-mentioned autoimmune and chronic inflammatory disorders, as well as allotransplant-associated immune-pathologies.

Our institute has extensive and long-standing expertise in conducting studies using non-human primates. (24-26). Different immunomodulatory therapies have been evaluated in the context of organ transplantation, RA and MS (24). There is also extensive experience with PK/PD studies that were performed in non-human primates in the context of these therapy evaluation studies (25). The investigators involved have extensive experience with analysis of immune responses, immune-cell phenotyping with particular knowledge on T_{REG} (27), analysis of cytokine responses and monitoring of well-being of the animals, including clinical chemistry and haematology evaluation. There is an in-house pathology department for pathology and immune-histochemical assessment.

3.3 Relevance

What is the scientific and/or social relevance of the objectives described above?

Medical. Autoimmune, autoinflammatory and transplantation-related pathologies comprise a diverse set of chronic diseases commonly featuring a dysregulation of the immune system with either overt activation of effector cells or reduced activity of regulatory cells. Such often chronic and non-curable diseases have an estimated prevalence of about 3-9% of the population. Treatment is generally based on broad immunosuppression affecting nonspecifically all immune cells, thereby causing significant long-term side effects, including increased susceptibility to severe infections and malignancies. Moreover, treatment efficacies of current immunosuppressive drugs are often insufficient, demonstrating the need for novel therapies. An increasing number of immunomodulatory mAb are either approved or in early-to-late clinical trials for the treatment of chronic inflammatory conditions, autoimmune diseases and organ transplant rejection (17). Their application has led to great improvements in the treatment of RA, psoriasis, psoriatic arthritis, ankylosing spondylitis, SLE, MS, Crohn's disease, ulcerative colitis (17). However, each therapy has its limitations and is effective against only certain diseases, while being ineffective or even harmful in other cases (17). This spectrum is mostly caused by the fact that most immunomodulatory mAb target specific molecules that are only implicated in certain diseases and not in others. There is a high unmet medical need to develop mAb therapies that are effective against a broader range of inflammatory diseases and could be used either as stand-alone or in support of current mAb therapies to improve efficacy and address problems of anti-idiotypic antibody formation that would lead to loss of efficacy.

Societal. The number of people with MS, RA, SLE, Crohn's disease and ulcerative colitis totals to around 300,000 in the Netherlands alone. Therefore, the societal impact of a broadly applicable therapy against these diseases is enormous. Furthermore, T_{REG} therapy can potentially be used to prevent type I diabetes mellitus, when applied in the early inflammatory stages of disease. Since, type I diabetes has life-long consequences and concerns about 100,000 people (in The Netherlands), the economic and societal implications of prevention are considerable.

Scientific. While the underlying principle of using IL-2 targeting mAb to amplify number and function of T_{REG} have already been established in mouse models (14), the results obtained in non-human primates, with mAb generated for use in humans, will be published.

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3.4 Research strategy

3.4.1 Provide an overview of the overall design of the project (strategy).

In this project proposal novel strategies to expand and enhance the function of T_{REG} *in vivo* will be evaluated in non-human primates as part of their pre-clinical development trajectory. Strategies are either based on novel stabilized forms of IL-2 or IL-2/mAb complexes that result in preferential activation and expansion of T_{REG} instead of immune-effector cells. Successful compounds with adequate PK/PD properties and no adverse effects will be tested further in animal models of human disease and clinical studies in human volunteers and patients, but these studies are not part of this project license application.

Criteria to select candidates for testing are:
- The therapeutic agent must have been tested in relevant cell cultures for absence of toxicity and *in vitro* efficacy
- The therapeutic agent, or a surrogate mAb binding to a homologous target in case of IL-2/mAb complexes, must have been evaluated in rodent models for immunomodulatory effect and absence of toxicity
- The evaluation in non-human primates is part of the pre-clinical evaluation of the therapeutic agent.

A single combined DRF PK/PD approach will be followed, in which animals will be injected with the therapeutic agent at one or multiple time points and blood is taken to assess drug levels, changes in immune cell number and phenotype, cytokine expression levels and clinical chemistry and hematology parameters. Two to three different doses of the drug will be tested in separate groups of animals, together with a negative control group. The primary study goal is to obtain a selective expansion and activation of T_{REG} cells over the other leucocyte subsets in the absence of adverse events.

3.4.2 Provide a basic outline of the different components of the project and the type(s) of animal procedures that will be performed.

The project consists of one type of experiment, namely the evaluation of the therapeutic agent in a DRF PK/PD approach as described under 3.4.1. The selection of the precise agent and doses tested depend on information obtained in preceding *in vitro* evaluation and studies in smaller animal models

3.4.3 Describe the coherence between the different components and the different steps of the project. If applicable, describe the milestones and selection points.

The proposal uses only one type of study.

3.4.4 List the different types of animal procedures. Use a different appendix 'description animal procedures' for each type of animal procedure.

Serial number	Type of animal procedure
1	DRF and PK/PD analysis of Treg inducing therapeutic agents in non-human primates
2	
3	
4	
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