# 1. Synopsis

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| **Name of Sponsor/Company:**<br>Centre for Human Drug Research<br>Zernikedreef 8<br>2333 CL Leiden<br>The Netherlands | |
| **Protocol number:** CHDR1701 | **Sponsor protocol number:** CHDR1701 |
| **Name of Finished Product:**<br>Tetanustoxoïd<br>Immucothel®/Alhydrogel® | **Name of Active Ingredients:**<br>Tetanustoxoïd >40 IU in 0.5 mL<br>Immunocyanine 0.1 mg/aluminium hydroxide gel 2% 0.9 mg in 0.5 mL |
| **Title of Study:**<br>A study to characterize the humoral and cellular response following simultaneous immunisation with a neo-antigen (KLH) and a recall antigen (tetanus) in healthy volunteers | |
| **Investigators**<br>M. Moerland, PhD (Principal Investigator)<br>J. Burggraaf, MD, PhD (Medical responsibility)<br>P. Gal, MD, PhD (Co-investigator)<br><br>**Report written by**<br>P. Gal, MD, PhD (Co-investigator)<br>M. Saghari, MD (Study physician) | |
| **Study Centre:**<br>Centre for Human Drug Research (CHDR)<br>Zernikedreef 8<br>2333 CL Leiden<br>The Netherlands | |
| **Publication (Reference):** Not applicable | |
| Studied Period:<br>February 2017 – May 2017 | Phase of Development:<br>0 |
| **Background and Rationale**<br>Keyhole limpet hemocyanin (KLH) is derived from the keyhole limpet, a type of mollusc. KLH is regarded as an ideal immunisation antigen for studying T-cell dependent immune response to a neo-antigen. KLH is available as a pure homogeneous substance. As a clinical grade product (KLH subunits) it has been used widely in patients and the injected form appeared to be associated with mild local reactions and perhaps mild pyrexia (Immucothel® SPC). It appeared to be immunogenic for the entire population, it had no cross-reacting antibody, and it elicited predictable primary T-cell dependent immune responses following one or two administrations.<br>For a future FIH trial investigating the effects of a novel drug blocking OX40L/OX40, a model is desirable that allows direct pharmacodynamics (PD) assessment of the compound. In this future study, both the influence of the compound on the immune response following presentation of a neo-antigen and of a recall antigen needs to be assessed. KLH will be the neo-antigen that will be administered. Tetanus toxoid will be used as recall antigen, since the entire population of the Netherlands born after 1957 has been immunized against tetanus.<br>The aim of the present study was to characterize the humoral and cellular response after neo-antigen presentation (KLH) and recall antigen presentation (tetanus toxoid), and evaluate if the T cell response to one antigen was substantially modulated by the response to the other antigen. Characterization and quantification of the immune response to these immunisations would allow rational design of the future OX40L/OX40 blocking study, and allow application of KLH and tetanus toxoid immunisations in other future clinical studies. | |
| **Objectives:**<br>Investigation of the immune response following immunisation with Immucothel®/Alhydrogel® with or without tetanus. Per efficacy endpoint, the following parameters were explored:<br>(a) Response size;<br>(b) Inter-individual variability of the response;<br>(c) Time course of the response.<br>Moreover, for each efficacy endpoint, it had to be confirmed that a simultaneous administration of tetanus | |

toxoid did not interfere (or only minimally) with the KLH response. This was considered valuable information to support simultaneous KLH/tetanus toxoid immunisations in the future intervention trial targeting OX40L/OX40. The data generated in the current study would allow selection of the most robust readout measures for quantification of the Immucothel®/Alhydrogel®-induced immune response in the future OX40L/OX40 study and allow for a power analysis of studies using this model.

**Methodology:**
This study had a randomized, double-blind, placebo-controlled design investigating the effects of immunisation with Immucothel®, adsorbed to Alhydrogel®, and tetanus toxoid administered to healthy male volunteers.

**Main Parameters:**
*Efficacy endpoints:*

- KLH-specific and tetanus toxoid-specific IgG and IgM titers (ELISA);
- Ex vivo lymphocyte proliferation upon an Immucothel® and tetanus toxoid challenge. Response quantification by Cell Trace Violet and BrdU and cytokine release (IL-2 and IFNγ);
- Ex vivo lymphocyte activation upon an Immucothel® and tetanus toxoid challenge. Response quantification by flow cytometric analysis of T cell activation markers (e.g. CD25, CD95, CD71, CD154);
- Erythema and swelling at the sites of intradermal KLH (Delayed Type Hypersensitivity (DTH) upon intradermal injection of 1 µg Immucothel® in 10µL, on the flexor aspect of the forearm).

*Tolerability / safety endpoints:*

- Treatment-emergent (serious) adverse events ((S)AEs)
- Concomitant medication
- Clinical laboratory tests
  - Haematology
  - Chemistry
  - Urinalysis
- Vital signs
  - Pulse Rate (bpm)
  - Systolic blood pressure (mmHg)
  - Diastolic blood pressure (mmHg)
- Electrocardiogram (ECG)
  - Heart Rate (HR) (bpm), PR, QRS, QT, QTcF

**Number of subjects** (planned and analysed): N = 15 (planned and analysed)

**Main Criteria for Inclusion and Exclusion:**
*Inclusion:*
Eligible subjects had to meet all of the following inclusion criteria at screening:

1. Healthy male subjects, 18 to 45 years of age (inclusive). The health status was verified by absence of evidence of any clinical significant active or uncontrolled chronic disease following a detailed medical history and a complete physical examination including vital signs, laboratory measurements and 12-lead ECG;
2. Body mass index (BMI) between 18 and 30 kg/m2, inclusive, and with a minimum bodyweight of 50 kg;
3. Anti-tetanus toxoid antibody titer ≥0.1 IU/mL
4. Willing to give written informed consent and willing and able to comply with the study protocol.

*Exclusion:*
Eligible subjects had to meet none of the following exclusion criteria at screening:

1. Any disease associated with immune system impairment, including auto-immune diseases, HIV, any confirmed history of severe allergic reaction and transplantation patients;
2. Known infection requiring antibiotic therapy within the last three months prior to the study
3. Previous known exposure to Immucothel® or KLH;
4. Any adverse immune reaction following immunisation with tetanus toxoid;
5. Known allergy against Thiomersal®, which is a stabilizer in the tetanus toxoid immunisation;

6. Received immunosuppressive or immunomodulatory medication within 30 days prior to enrolment or planned to use during the course of the study;
7. Use of medication (prescription or over-the-counter) within 21 days of the first study day, or less than 5 half-lives (whichever is longer), and during the course of the study;
8. Participation in an investigational drug or device study within 3 months prior to screening or more than 4 times a year;
9. Previous participation in an investigational drug or device study involving the dosing of a biological targeted at any immune pathway within one year prior to screening;
10. Loss or donation of blood over 500 mL within three months prior to screening;
11. Any (medical) condition that would, in the opinion of the investigator, potentially compromise the safety or compliance of the subject or may preclude the subjects' successful completion of the clinical trial.
12. History of Schistosomiasis (infection with Schistosoma parasite).
13. History or current nicotine use in access of 5 cigarettes per day, or unable not to smoke during the course of the study, defined as between the screening visit and the final visit.
14. Tetanus toxoid immunisation within the last 10 years before study participation

**Test Product, Dose and Mode of Administration:**
Investigational drug

- Immucothel® (Biosyn) 0.1 mg/Alhydrogel® 2% (Brenntag) 0.9 mg dissolved in 0.5 mL for intramuscular injection in the left deltoid muscle
- Tetanus toxoid (Bilthoven Biologics) >40 IU dissolved in 0.5 mL for intramuscular injection in the right deltoid muscle
- Immucothel® (Biosyn) 0.001 mg dissolved in 0.1 mL for intradermal injection in the ventral left forearm

**Duration of Treatment:** 2 immunisations in left and right deltoid muscle

**Reference Therapy, Dose and Mode of Administration:**
Comparative treatment formulation

- Placebo (saline) of 0.5 mL for intramuscular injection in the deltoid muscle(s)

**Statistical Methods:**
For each efficacy endpoint, the response was quantified by descriptive statistics (average, median, min-max). The variability of the response was calculated. These data (response size and variability) were used as basis for power calculations for future intervention trials, providing minimal detectable effect sizes. As such, each efficacy endpoint was qualified for its feasibility as pharmacodynamic endpoint in the future intervention trial that has a sample size of six subjects per cohort.

**Results and Discussion**
Participation and demographics
38 subjects were screened of which 15 subjects were enrolled into the trial and randomized to 1 of the 3 treatment arms. Demographics and baseline characteristics were comparable across the treatment groups. The analysis populations consisted of 15 subjects for both the Intent-to-treat (ITT) and the Clinical Evaluable population. Treatments were administered as per protocol. All dosed subjects completed the study.
KLH-specific and tetanus toxoid-specific IgG and IgM titers showed significant differences among treatment groups, as was expected based on the available literature. Proliferation assays, surface markers and ex vivo cytokine release were all not different between treatment groups. For the DTH test, the Antera camera and LSCI were able to detect a difference between treatment groups, where other modalities did not detect such a difference.
In terms of safety, the administered treatments were well-tolerated, without AEs considered related to the treatment.

**Conclusions**
The administration of KLH and tetanus toxoid is a safe and well-tolerated treatment and induces a quantifiable immunological response. Selected elements of the study have the potential to serve as a model to detect the pharmacodynamics effects of compounds aimed at immunological modulation.