STUDY PROTOCOL

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| **STUDY TITLE:** | A Phase I, Double-blind, Randomised, Single Centre, Parallel-group, Single Ascending Dose, Placebo-controlled Study of the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of CpG ODN D35 after Subcutaneous Administration in Healthy Male Subjects |
| **STUDY NUMBER:** | RD 777/35000 (DNDi‐CpG‐01) |
| **EudraCT NUMBER:**  **IRAS ID:** | 2021-001179-18  297265 |
| **INVESTIGATIONAL MEDICINAL PRODUCT(s):** | CpG ODN D35 |
| **PLANNED STUDY DOSES:** | Cohort 1: SC injection of 7.5 mg of CpG ODN D35 or placebo  Cohort 2: SC injection of 22.5 mg of CpG ODN D35 or placebo  Cohort 3: SC injection of 67.5 mg of CpG ODN D35 or placebo  Cohort 4 (optional): SC injection of 180 mg of CpG ODN D35 or placebo |
| **PRINCIPAL INVESTIGATOR:** | Dr Annelize Koch Simbec-Orion  Merthyr Tydfil CF48 4DR  United Kingdom |
| **STUDY SPONSOR:** | Drugs for Neglected Diseases initiative (DNDi), Chemin Camille Vidart, 15  1202 Geneva  Switzerland |
| **SPONSOR’S LEGAL REPRESENTATIVE:** | Orion LR Limited  Merthyr Tydfil CF48 4DR  United Kingdom |
| **SPONSOR’S RESPONSIBLE PHYSICIAN:** | Dr Byron Arana  DNDi  Chemin Camille Vidart, 15  1202 Geneva  Switzerland |

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| **STUDY MONITOR:** | | Clinical Operations, Simbec-Orion Clinical Development, Simbec-Orion  Merthyr Tydfil CF48 4DR  United Kingdom*.* |
| **ADDITIONAL DEPARTMENTS:** | |  |  | | --- | --- | | Pharmacovigilance Department: | DNDi  Chemin Camille Vidart, 15  1202 Geneva  Switzerland  Tel: +41 (0) 78 613 39 30 | | Pharmacovigilance Provider (Third Party): | United BioSource Corporation (UBC)  16 Chemin des Coquelicots  1214 Vernier  Switzerland  Tel: +41 (0) 22 59 64 42 0 | | Bioanalytical Laboratory: | Oncodesign  25-27 avenue du Québec  91140 Villebon-sur-Yvette  France  Tel: +33 3 80 78 82 60 | | **PBMC sample Analysis:** | Prof. Ken Ishii  University of Tokyo  4-6-1 Shirokanedai, Minato-ku  Tokyo 108-8639  Japan  Tel: +81 35 449 5314 | | Data Protection Representative: | Mydata - T LTD  Waldeck House, Lyne Lane, Chertsey, KT16 0AW  United Kingdom dndi.dpr@mydata-trust.info | | |

PROTOCOL FINALISATION STATEMENT

This protocol is not considered final unless accompanied by an approval letter from the Research Ethics Committees and Notice of Acceptance from the relevant Competent Authority.

Protocol Prepared by: LT

# SIGNATURE PAGE

I declare that I have read and understood this study protocol. I agree to abide by this protocol (subject to any amendments agreed in writing between the Sponsor and Principal Investigator). Any changes in procedure will only be made, if necessary, to protect the safety, rights or welfare of the participants.

**STUDY SPONSOR:**

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**PRINCIPAL INVESTIGATOR:**

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# PROTOCOL AMENDMENT/REVISION HISTORY

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| **Protocol Version/**  **Date** | **Type of Amendment** | **Amendment Rationale** | **Sections Affected** | **Summary of Amendment / Changes to the Protocol** |
| V1.0/19 March 2021 | N/A | N/A | N/A | N/A |
| V2.0/15 April 2021 | N/A | Response to Grounds for Non-Acceptance (GNA) – MHRA | Synopsis  10.5.1 Contraception  10.6.7.3 Dose Escalation Increments  10.7.1 Efficacy and Safety Measurements Assessed and Flow Chart  10.7.5 Appropriateness of Measurements | Additional assessments: -Day 4 brief physical exam -Day 4 12-Lead ECG  -Day 7 Laboratory safety assessments  Amended text relating to use of a condom when engaging in sexual intercourse.  Text to state that the maximum clinical dose will not exceed 180 mg unless approved by REC and MHRA in a substantial amendment.  Additional assessments added to Table 5 and footnote wording amended: -Day 4 brief physical exam -Day 4 12-Lead ECG  -Day 7 Laboratory safety assessments  Table 6 edited to include additional laboratory blood samples and total blood volume amended. |
| V3.0/10 May 2021 | Substantial amendment | Response to Provisional Opinion from REC | Signature Page Synopsis  Synopsis & Section 10.2.2 (Trial Stopping Criteria)  Synopsis & Section 10.2.3 (Dose Escalation Stopping Criteria)  Synopsis & Section 10.4.1 (Inclusion Criteria)  Synopsis & Section 10.4.2 (Exclusion Criteria)  Section 10.6.1 (Identity)  Synopsis & Section 10.6.4 (Administration)  Synopsis & Section 10.7.1 (Efficacy and Safety Measurements Assessed and Flow Chart)  Section 10.7.2 (Demographics and Background Assessments)  Section 10.7.5 (Appropriateness of Measurements)  Synopsis & throughout protocol  Section 11.2 (Protocol Amendment) | Address of Statistician.  Added ‘Follow-up visit (Approximately 6 weeks for each individual, from the screening to post study follow-up).’  ‘The study will resume after review and approval of an appropriate substantial amendment by the MHRA and REC after review and approval of an appropriate substantial amendment.’ has been reworded to ‘If any of the above criteria are fulfilled, dose escalation will only proceed once an appropriate substantial amendment has received regulatory approval from the MHRA and ethical approval from the ethics committee associated with the study.’  Added ‘Proposed maximum clinical dose of 180 mg will not be exceeded in this study. If the Sponsor determines that it is appropriate and necessary to exceed the current planned maximum dose of 180 mg, this will not be implemented until a substantial amendment has been submitted and receives approval from both the REC and MHRA.’  Amended to ‘Dosing will be temporarily stopped (via an initial email notification to Research Ethics Committee (REC)/Medicines and Healthcare products Regulatory Agency (MHRA) and then a temporary halt substantial amendment) pending evaluation of all available data if any of the following criteria are fulfilled:  If any of the above criteria are fulfilled, dose escalation will only proceed once an appropriate substantial amendment has received regulatory approval from the MHRA and ethical approval from the ethics committee associated with the study.’  Systolic blood pressure in inclusion criterion 4 has been amended to ‘blood pressure between ≥ 100 and ≤ 140 mmHg’.  Exclusion criteria 20 ‘Users of nicotine products i.e., current smokers or ex-smokers who have smoked within the 6 months prior to first dose administration the study medication or users of cigarette replacements (i.e., e-cigarettes, nicotine patches or gums).’ was changed to ‘Users of nicotine products i.e., current smokers or ex-smokers who have smoked within the 6 months prior to Screening or users of cigarette replacements (i.e., e-cigarettes, nicotine patches or gums).  This progression of doses is provided for information. A maximum increase of 3-fold in between cohorts is envisaged based on the dose/concentration-response curves observed in in vitro and in vivo models. This will be decided by the SRC at the end of each cohort.  Amended to ‘The average fill volume in a vial is 2.5 mL and the injection volume will be 2.3 mL or less per SC injection site. For doses requiring a volume above the 2.3 mL, or in case the PI considers more appropriate to decrease the volume per injection site due to feasibility constraints, additional injections in different locations can be used for administration.’  Removal of Day 1 pre-dose laboratory safety tests (biochemistry including C-reactive protein, haematology, coagulation, urinalysis and urine microscopy, if needed).  Added ‘ethnicity’ in demographic data.  Blood volumes were updated in Table 6 (Summary of Blood Volume) and included 5 mL ESR sample at all haematology timepoints in Table 6.  Baseline for laboratory safety data has been changed to Day -1.  Added ‘Increase in dose above 180 mg would be considered substantial.’  Administrative changes were made throughout the main text of the protocol to be consistent with the synopsis. |
| V4.0/23 June 2021 | Non-substantial amendment | Protocol clarification | Synopsis (Post Study Follow-up (Day 14 ± 1 Day)  Section 4. Table of Contents  Section 5, Abbreviations used in the Protocol Text  Section 8.4 (Risk-Benefit Assessment)  Section 10.6.11 (Emergency Treatment) | Added ‘Injection site examination’  Updated.  Additional abbreviations added.  Last paragraph has been amended to ‘Coagulation parameters, including aPTT, will be monitored as a standard assessment in the study, and complement evaluation will be triggered in case of ~~emergency~~ fever above 38.5°C (see Section 10.6.11).’  The following paragraph has been amended from ‘Above 38.5°C, blood specimens should be collected to monitor the immune activation: interleukin-6, complement (CH50, C4, C5a, and Bb fragments and assayed as soon as possible).’ to ‘*For temperatures reported/recorded above 38.5°C, the following blood specimens will be collected as soon as possible to monitor the immune activation: interleukin-6 and complement samples (CH50, C4, C5a, and Bb fragments)*.’  The following paragraphs have been added:   * Samples for the measurement of interleukin-6 in addition to CRP (as detailed below) will be sent for analysis to an appropriate laboratory and results available within a suitable timeframe in order to determine appropriate management, treatment and care for the participant as critical markers for early indicators of CRS. * Analysis of complement samples will be undertaken by an appropriate laboratory. The outcome of complement analysis will not be utilised for the purposes of determining appropriate management, treatment and care for the participant; however, results will be used for further understanding of the mechanisms by which CRS may be triggered in the context of this IMP. Therefore, it is not required that results of complement analysis are available to determine participant treatment in the event of potential CRS.   Administrative changes were made throughout protocol.  Update to protocol template v10.0 (28 May 2021) with more abbreviations added and Table 7 updated. |

# SYNOPSIS

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| **NAME OF company:** DNDi | |
| **NAME OF INVESTIGATIONAL MEDICINAL PRODUCT:** CpG ODN D35 | |
| **NAME OF ACTIVE INGREDIENT:** Oligodeoxynucleotide (ODN) | |
| **TITLE of STUDY**: A Phase I, Double-blind, Randomised, Single Centre, Parallel-group, Single Ascending dose, Placebo-controlled Study of the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of CpG ODN D35 after Subcutaneous Administration in Healthy Male Subjects | |
| **principal INVESTIGATOR:** Dr Annelize Koch | |
| **Study centre:** Simbec-Orion Clinical Pharmacology  Merthyr Tydfil, CF48 4DR, UK | |
| **CLINICAL PHASE:** I (First in Human) | |
| **OBJECTIVES:**  **Primary Objective**   * To assess the safety and tolerability of a single subcutaneous dose of CpG ODN D35 in healthy male subjects.   **Secondary Objectives**   * To determine PK parameters of CpG ODN D35 in plasma after single subcutaneous dose in healthy male subjects.   **PD and Exploratory Objectives**   * To investigate changes from baseline levels of serum cytokine and chemokine parameters (CXCL10, IFN-γ, IL-6, optional: IFN-α, MIP1α, IL-10, TNFα and/or other parameters) after a single subcutaneous dose of CpG ODN D35. * To investigate changes of mRNA markers after a single subcutaneous dose of CpG ODN D35, by exploratory analysis of cytokine and chemokine gene expression. * To investigate immunological markers in Peripheral Blood Mononuclear Cells (PBMC) isolate (for example CXCL10, Mx1, CD80, OAS1, IRF7, IFI1 and/or other parameters). | |
| **METHODOLOGY:**  The study is a Phase 1 single centre, double-blind, randomised, placebo-controlled, parallel-group, single ascending dose study of CpG ODN D35 administered subcutaneously in healthy male subjects aged between 18 and 50 years.  The study will consist of up to 4 cohorts of 8 male subjects (cohort 4 is optional). Subjects will be randomly assigned to receive a single subcutaneous (SC) dose of CpG ODN D35 (6 subjects) or placebo (2 subjects) in a sequential escalating manner.  Each cohort will follow a sentinel dose escalation schedule.   * Two (2) subjects will be dosed in each sentinel group (1 subject on active investigational medicinal product (IMP) and 1 subject on placebo). * The 6 remaining subjects will be randomized and split into two sub-groups with 3 subjects in each sub-group. The decision to proceed with the administration of the first sub-group of subjects will be taken by the Investigator based on clinical and biological safety data after at least a 72-hour observation period of the sentinel subjects. The 3 subjects from the second sub-group will be dosed upon Investigator assessment after approximately a 24-hour observation period of the first subject of the first sub-group. * At the end of each dose level, an interim safety report will be issued by the Investigator, for review by the safety review committee (SRC). No more than 3 subjects will be administered CpG ODN D35 on each day. A minimum interval of 30 minutes between dosing of any 2 subjects will be ensured (not required for sentinels).   Each cohort will follow a Screening Period, Treatment Period and Post Study Follow-up visit (Approximately 6 weeks for each individual, from the screening to post study follow-up).  **Screening Period (Day -28 to Day -2):**  After signing the informed consent form (ICF), Screening assessments will be performed within 28 days of the planned dose to ensure the eligibility of participants. Screening assessments will include:   * Medical and surgical history * Demographic data * Hepatitis/human immunodeficiency virus (HIV) serology * Inclusion/Exclusion Criteria * Weight and height/body mass index (BMI) * Vein assessment * Urine drugs of abuse (DOA) and alcohol/cotinine screen * Physical examination (full) * Vital signs (supine systolic and diastolic blood pressure, pulse rate, oral temperature and respiration rate) * Laboratory safety tests (biochemistry including C-reactive protein, haematology, coagulation, urinalysis and urine microscopy, if needed) * Thyroid stimulating hormone * 12-lead electrocardiogram (ECG) * Adverse event (AE) * Serious Adverse Event (SAE) * Prior and concomitant medication   **Treatment Period (Day -1 to Day 7):**  Subjects will be admitted to the clinic on the morning of Day -1 and will remain in the unit until discharge on Day 4 (72 hours post-dose) when all scheduled assessments and procedures have been performed. All subjects will come back on Day 7 for a return-visit.  **Day -1:**   * + Medical and surgical history (update)   + Polymerase chain reaction (PCR) test for COVID-19   + Inclusion/exclusion criteria   + Weight   + Vein assessment   + Urine DOA and alcohol/cotinine screen   + A brief physical examination   + Vital signs (supine systolic and diastolic blood pressure, pulse rate, oral temperature and respiration rate)   + Laboratory safety tests (biochemistry including C-reactive protein, haematology, coagulation, urinalysis and urine microscopy, if needed)   + 12-lead ECG   + AE   + SAE   + Prior and concomitant medication   **Day 1:**  Dose administrations of CpG ODN D35 or placebo will occur in the morning of Day 1 in a randomised, double-blind manner.  The following procedures will be performed on **Day 1 pre-dose**:   * + Randomisation   + A brief physical examination   + Injection site examination   + Vital signs (supine systolic and diastolic blood pressure, pulse rate, oral temperature and respiration rate)   + 12-lead ECG (triplicate)   + AE   + SAE   + Prior and concomitant medication   + PD blood sample for cytokines and chemokines   + PK plasma sample for CpG ODN D35   + Blood sample for mRNA markers   + Blood sample for PBMC isolate   + Blood sample for titration of autoantibodies   Subjects will receive a SC dose of CpG ODN D35 or placebo in the morning.  The following procedures will be performed on **Day 1, after dosing:**   * + A brief physical examination: 2 h, 4 h and 8 h post-dose   + Injection site examination: 2 h, 4 h and 8 h post-dose   + Vital signs (supine systolic and diastolic blood pressure, pulse rate, oral temperature and respiration rate): 15 min, 30 min, 1 h, 2 h, 4 h, 8h and 12 h post-dose   + 12-lead ECG: 15 min, 30 min, 1 h and 8 h post-dose   + AE   + SAE   + Prior and concomitant medication   + PD blood sample for cytokines and chemokines: 8 h and 12 h post-dose   + PK plasma sample for CpG ODN D35: 10 min, 20 min, 30 min, 45 min, 60 min, 2 h and 4 h post-dose   + Blood for mRNA markers: 4 h, 8 h and 12 h post-dose   **Day 2:**   * + A brief physical examination   + Injection site examination   + Vital signs (supine systolic and diastolic blood pressure, pulse rate, oral temperature and respiration rate)   + Laboratory safety tests (biochemistry including C-reactive protein, haematology, coagulation, urinalysis and urine microscopy, if needed)   + AE   + SAE   + Prior and concomitant medication   + PD blood sample for cytokines and chemokines   + Blood sample for mRNA markers   + Blood sample for PBMC isolate   **Day 3:**   * + Vital signs (supine systolic and diastolic blood pressure, pulse rate, oral temperature and respiration rate)   + AE   + SAE   + Prior and concomitant medication   + PD blood sample for cytokines and chemokines   + Blood sample for mRNA markers   **Day 4:**   * + A brief physical examination   + Injection site examination   + Vital signs (supine systolic and diastolic blood pressure, pulse rate, oral temperature and respiration rate)   + Laboratory safety tests (biochemistry including C-reactive protein, haematology, coagulation, urinalysis and urine microscopy, if needed)   + 12-Lead ECG   + AE   + SAE   + Prior and concomitant medication   + Issuing temperature diary (to record oral temperature in the morning and evening at home from Day 4 evening to Day 6 evening)   If all assessments are satisfactory to the PI (or deputy), subjects will be discharged from clinic after all Day 4 procedures are completed.  **Day 7 (Return-visit):**   * + A brief physical examination   + Injection site examination   + Vital signs (supine systolic and diastolic blood pressure, pulse rate, oral temperature and respiration rate)   + Laboratory safety tests (biochemistry including C-reactive protein, haematology, coagulation, urinalysis and urine microscopy, if needed)   + AE   + SAE   + Prior and concomitant medication   + PD blood sample for cytokines and chemokines   + Blood sample for PBMC isolate   + Collect previous temperature diary (Day 4 - Day6)   + Issue new temperature diary (to record oral temperature in the morning and evening at home from Day 7 evening to evening before post study)   **Post Study Follow-up (Day 14 ± 1 Day):**  A post study follow-up visit will take place on Day 14 (± 1 Day) to ensure the ongoing wellbeing of the subjects.   * + Weight   + A brief physical examination   + Injection site examination   + Vital signs (supine systolic and diastolic blood pressure, pulse rate, oral temperature and respiration rate)   + Laboratory safety tests (biochemistry including C-reactive protein, haematology, coagulation, urinalysis and urine microscopy, if needed)   + Thyroid stimulating hormone   + AE   + SAE   + Prior and concomitant medication   + Collecting temperature diary (Day 7 - Day 12/13 – dependent on whether post study is performed on Day 13 or Days 14/15)   If all follow-up assessments are satisfactory to the PI (or deputy), the subject will be discharged from the study. If any AEs are ongoing, or any assessments are not satisfactory, subjects may be recalled to the unit for follow-up assessments until the PI/deputy is satisfied the subject may be discharged from the study. Subjects will be advised to return or contact the unit at any time if they think they may be experiencing any AEs. | |
| **DOSE ESCALATION AND STOPPING CRITERIA**  For dose escalation to proceed, data from the preceding dose level must be available from a minimum of 6 evaluable subjects who have completed the planned safety assessments up to Day 4 (72h after dosing) and all planned PK assessments after dosing to ensure at least 4 subjects had received active IMP.  All available safety data, including follow-up data from lower/previous dose cohorts, will be reviewed at the time of dose-escalation. Subjects will be deemed as evaluable for dose escalation purposes if they have received theplanned study dose and had sufficient plasma samples collected to estimate, if possible Cmax and AUClast irrespective of whether they have received active or placebo treatment.  The decision must be documented in writing by the SRC at the dose escalation review meeting (DERM) before the next escalated dose level is administered to volunteers.  Safety parameters will be graded according to the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials[01].  **Trial Stopping Criteria**  Dosing will be temporarily stopped (via an initial email notification to Research Ethics Committee (REC)/Medicines and Healthcare products Regulatory Agency (MHRA) and then a temporary halt substantial amendment) pending evaluation of all available data if any of the following criteria are fulfilled:   * A serious adverse reaction (SAR) (i.e., a SAE considered at least possibly related to CpG ODN D35) in one subject, or * ‘Severe’ non‐serious adverse reactions (AR) (i.e., severe non‐serious adverse events) considered as, at least, possibly related to CpG ODN D35 in two subjects in the same cohort, independent of within or not within the same system‐organ class. * Any other event deemed to pose an unacceptable risk to individuals by the PI.   If, after an internal safety review, it is appropriate to restart the trial, a substantial amendment will be submitted to the MHRA and Ethics Committee. The trial will not restart until the amendment has been approved by MHRA and Ethics Committee.  **Dose Escalation Stopping Criteria**  A dose level will not be repeated, or increased, if the results of safety tests give the Sponsor or Investigator cause for concern, or if:   * Any of the trial stopping criteria are reached (see above). * There are clinical indications that could suggest a moderate/severe documented cytokine release syndrome \*   \* Cytokine release syndrome (CRS) can present from mild flu-like syndrome, fever, fatigue, headache, cough, tachypnoea, rash, arthralgia, myalgia, hypotension or high fever and can progress to an uncontrolled systemic inflammatory response with vasopressor-requiring circulatory shock, severe dizziness and confusion, vascular leakage, disseminated intravascular coagulation, multi-organ system failure, acute respiratory distress syndrome, renal failure or signs of cardiac dysfunction with reduced ejection fraction on ultrasound, vascular leakage with peripheral and/or pulmonary oedema. Laboratory abnormalities that are common in patients with CRS include cytopenias, elevated creatinine and liver enzymes, deranged coagulation parameters and a high c-reactive protein (CRP).   * + Two of the subjects report severe flu-like symptoms, high fever, fatigue, headache, cough, tachypnoea, rash, arthralgia, myalgia or hypotension and considered at least possibly related to IMP.   + Or if one subjects suffers from circulatory shock, severe dizziness and confusion, vascular leakage, disseminated intravascular coagulation, multi-organ system failure or acute respiratory distress syndrome and considered at least possibly related to IMP.   Or if 2 subjects have laboratory abnormalities such as cytopenias, elevated creatinine and liver enzymes, altered coagulation parameters, or elevated CRP levels: AST or ALT > 3ULN (without CPK increase) or ALP > 1.5 ULN or total bilirubin > 1.5 ULN, serum creatinine > 1.5 ULN, platelet count below 100 10^9/L, , lymphocyte count below 0.5 10^9/L, CRP >20 mg/L, and considered related to the IMP and confirmed 24 to 48 hours later.   * If the dose in a single subject is anticipated to exceed an AUClast of ≥ 755 ng\*h/mL. These limits are based on the exposure observed at the 15.9 mg/kg in cynomolgus monkeys (systemic NOAEL: AUClast of 755.8 ng\*h/mL in males). * If the dose in a single subject is anticipated to exceed a Cmax of ≥ 1230 ng/mL. These limits are based on the exposure observed at the 15.9 mg/kg in cynomolgus monkeys (systemic NOAEL: Cmax of 1230.7 ng/mL in males). * If 2 subjects have grade 3 or above injection site reaction which are related to IMP injections. * The Investigator considers the dose level to be not well tolerated.   If any of dose escalation stopping criteria are met, dosing will be halted and the SRC will be convened to explore whether it is reasonable to continue the study All SAEs, if any, will be reported to the MHRA as per guidance.  If any of the above criteria are fulfilled, dose escalation will only proceed once an appropriate substantial amendment has received regulatory approval from the MHRA and ethical approval from the ethics committee associated with the study.  Planned doses may be modified following a review of emerging data. Proposed maximum clinical dose of 180 mg will not be exceeded in this study. If the Sponsor determines that it is appropriate and necessary to exceed the current planned maximum dose of 180 mg, this will not be implemented until a substantial amendment has been submitted and receives approval from both the REC and MHRA.  Dose escalation will be dependent upon the accrual of acceptable safety (and PK) data. If it is not appropriate to escalate the dose according to the proposed dose escalation schedule, then the same dose (only where no dose escalation stopping rules have been met), an intermediate dose or a lower dose may be given following discussion between the Sponsor and the PI (or deputy). The timing, type and number of safety, PK and PD assessments may be modified. The number and/ or volume of blood samples per assessment may be increased as long as the total volume of blood drawn for a participant does not exceed 10% of the pre-specified total blood volume or surpass 500 mL (except when extra blood samples need to be taken for safety reasons). | |
| **number of PARTICIPANTs:**   * 4 cohorts of 8 healthy male subjects (cohort 4 is optional).   Replacement of subjects is acceptable in case of protocol violations, or withdrawal for personal reasons. Subjects withdrawn for safety reasons after dosing will not be replaced. | |
| **main INCLUSION criteria:**   1. Male healthy subjects 18 to 50 years old at the time of obtaining the informed consent. 2. Body weight ≥ 60 kg to ≤ 90 kg, BMI 18 to 30.1 kg/m2. BMI = body weight (kg) / [height (m)]2 3. Provision of written informed consent to participate as shown by a signature on the participant information sheet and consent form, after reading the information sheet and consent form, and after having the opportunity to discuss the trial with the Investigator or his/her delegate. 4. Normal blood pressure: Systolic blood pressure between ≥100 and ≤140 mmHg, Diastolic blood pressure ≤ 90 mmHg, measured after 10 min rest in supine position at Screening, admission, and pre-dose. 5. A resting heart rate (HR) between ≥45 and ≤90 bpm measured after 10 min rest in supine position at Screening, admission, and pre-dose. 6. ECG recording without clinically significant abnormality, including a QTcF measure of ≤ 450 msec. 7. Male participant (and partner of childbearing potential) willing to use a highly effective method of contraception (see [Section 10.5.1](#_Contraception)), if applicable (unless anatomically sterile or where abstaining from sexual intercourse is in line with the preferred and usual lifestyle of the participant) from first dose until 3 months after last dose of IMP. 8. No clinically significant history of previous allergy / sensitivity to CpG ODN D35 or any of the excipients contained within the IMP(s). 9. No clinically significant abnormal test results for serum biochemistry, haematology and/or urine analyses within 28 days before the first dose administration of the IMP. 10. Participant with a negative urinary drugs of abuse (DOA) screen (including alcohol/cotinine) test results, determined within 28 days before the first dose administration of the IMP (N.B.: A positive test result may be repeated at the Investigator’s discretion). 11. Participant must be available to complete the study (including all follow-up visits). 12. Participant must satisfy an Investigator about his fitness to participate in the study. | |
| **MAIN Exclusion CRITERIA:**   1. Behavioral, cognitive, or psychiatric disease that, in the opinion of the Investigator, affects the ability of the participant to understand and cooperate with the study protocol. 2. History of clinically significant cardiovascular, renal, hepatic, neurological (especially seizures), immunological, psychiatric, myopathies, bleeding tendency, respiratory and particularly GI disease, especially peptic ulceration and chronic gastritis, GI bleeding, ulcerative colitis, Crohn’s Disease or Irritable Bowel Syndrome, as judged by the Investigator. 3. Individual or family history of pre-existing autoimmune or antibody-mediated diseases including (but not limited to): systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, Sjögren's syndrome, type 1 diabetes mellitus, auto-immune thyroiditis, Basedow syndrome, autoimmune thrombocytopenia; or proteinuria (greater than trace protein on urine dipstick testing). 4. History of allergy, hay fever, intolerance or photosensitivity to any drug or have a history of serious allergy, asthma, allergic skin rash or sensitivity to any drug. 5. Subjects who are taking, or have taken, any prescribed or over-the-counter drug (including non-steroidal anti-inflammatory drugs (NSAID)) in the 28 days or 5 half-lives (whichever is longer) before IMP administration. Administration of up to 3 g of paracetamol per day within 7 days of IMP administration is allowed. 6. Subjects who have received any prophylactic vaccine (including COVID-19 vaccine) or immunization within the last 28 days or use of corticosteroids or immunosuppressive drugs within 28 days of IMP administration. 7. Subjects with febrile illness or infectious illness within 2 weeks of IMP administration. 8. Subjects with positive hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (HCV Ab) and or human immunodeficiency virus (HIV) tests results at Screening. 9. Positive RT-PCR COVID-19 test at admission. 10. Donation or loss of greater than 500 mL of blood within the previous 3 months prior to IMP administration. 11. Major surgery within 12 weeks prior to Screening. 12. Subjects who are known or suspected alcohol abusers (more than 14 units of alcohol per week, one unit = 8 g or about 10 mL of pure alcohol). Positive alcohol test at Screening or admission. 13. Demonstrating excess in caffeine/xanthine consumption (more than 6 cups of coffee or equivalent a day). 14. History of use of drugs of abuse in the past 2 years. 15. Subjects who do not have suitable veins for multiple venepunctures/cannulation. 16. Subjects who have any clinical condition or prior therapy which, in the opinion of the Investigator, could jeopardize the safety or rights of a volunteer participating in the trial or would render them unable to comply with the protocol. 17. Participation in a non-marketed drug clinical study within 3 months or five half-lives (whichever is longer) or a marketed drug clinical study within 30 days or five half-lives (whichever is longer) before the first dose of IMP (washout period between studies is defined as the period of time elapsed between the last dose of the previous study and the first dose of the next study). 18. Subjects who are study site employees, or immediate family members of a study site or sponsor employee. 19. Inability to communicate well with the Investigators (i.e., language problem, poor mental development, or impaired cerebral function). 20. Users of nicotine products i.e., current smokers or ex-smokers who have smoked within the 6 months prior to Screening or users of cigarette replacements (i.e., e-cigarettes, nicotine patches or gums). | |
| **IMP administration:**  The investigational product will be supplied as a solution for SC injection in a sterile 2R Type I vial sealed with ETFE coated bromobutyl stopper and aluminium overseal. Each vial contains 15 mg/mL of CpG ODN D35 as a 2.5 mL single-use solution. The investigational product also contains non-active ingredient trehalose dihydrate (88 mg/mL) as a tonicity agent.  A placebo for CpG ODN D35 solution for injection will be provided which includes the same non-active excipient trehalose dihydrate (88 mg/mL) as the investigational product. The placebo has no obvious coloration whereas the CpG ODN D35 solution 15 mg/mL has a slight yellow tinge. Therefore, syringes used to administer the drug product to the subjects will be covered prior to their presentation to the Investigator to ensure blinding in clinical trials.  DNDi, Investigators and subjects will be blinded to treatment allocation. The site pharmacist and the analyst at bioanalytical laboratory will be unblinded to treatment allocation.  The planned doses are presented below:   * Cohort 1: SC injection of 7.5 mg of CpG ODN D35 (6 subjects) or placebo (2 subjects) * Cohort 2: SC injection of 22.5 mg of CpG ODN D35 (6 subjects) or placebo (2 subjects) * Cohort 3: SC injection of 67.5 mg of CpG ODN D35 (6 subjects) or placebo (2 subjects) * Cohort 4 (optional): SC injection of 180 mg of CpG ODN D35 (6 subjects) or placebo (2 subjects)   This progression of doses is provided for information. A maximum increase of 3-fold in between cohorts is envisaged based on the dose/concentration-response curves observed in *in vitro* and *in vivo* models. This will be decided by the SRC at the end of each cohort.  A single SC injection of IMP or placebo will be administered on the morning on Day 1, according to the randomisation, in semi-supine position after at least 8 hours fasting.  The average fill volume in a vial is 2.5 mL and the injection volume will be 2.3 mL or less per SC injection site. For doses requiring a volume above the 2.3 mL, or in case the PI considers more appropriate to decrease the volume per injection site due to feasibility constraints, additional injections in different locations can be used for administration. The location of injection is important for subcutaneous injections. The drug needs to be injected into the fatty tissue just below the skin. Some areas of the body have a more easily accessible layer of tissue, where a needle injected under the skin will not hit muscle, bone, or blood vessels. The most common injection sites are:   1. Abdomen: at or under the level of the belly button, about two inches away from the navel 2. Arm: back or side of the upper arm. 3. Thigh: front of the thigh.   The dosing will potentially necessitate several successive injections in different injection sites, given sequentially as soon as possible within a maximum of 3 minutes on Day 1. | |
| **STUDY VARIABLES/ENDPOINTS:**  **Primary Endpoints**   * Number and severity of treatment related adverse events, safety laboratory parameters, vital signs, physical examination including injection site examination, ECG parameters and injection site reactions.   + Systemic adverse events (including, but not limited to: fever, chills, headache, muscle aches, fatigue, nausea, vomiting, diarrhea, and/or joint pain).   + Safety laboratory parameters (biochemical and haematological parameters, C-reactive protein, urinalysis and coagulation).   + Vital signs (supine heart rate, systolic and diastolic blood pressure, oral temperature, respiration rate).   + Physical examination and injection site examination.   + 12-lead ECG (HR, RR, PR, QRS, QT, QTcF).   + Injection site reactions (redness, swelling, warmth, tenderness and/or pain with arm movement).   **Secondary Endpoints**  PK blood samples will be taken on Day 1 pre-dose and 10 min, 20 min, 30 min, 45 min, 60 min, 2 and 4 hours post-dose. Plasma CpG ODN D35 concentrations will be measured using a LC-MS/MS assay method**.**  The following PK parameters will be derived from plasma CpG ODN D35 concentrations**:**   * Cmax (ng/mL): Observed maximum plasma concentration. * Tmax (h): first time to reach Cmax. * λz (1/h): apparent first order terminal elimination rate constant. * t1/2 (h): plasma elimination half-life. * AUClast (ng.h/mL): AUC from 0 to the time of the last quantifiable concentration. * AUCall (ng.h/mL): AUC from 0 to the time of the last observation, regardless of whether the last concentration is measurable or not. * AUC0-inf (ng.h/mL): AUC extrapolated to infinity. * AUC% extrapolated (%): Residual area.   **PD and Exploratory Endpoints**   * PD endpoints:   + Explore changes from baseline levels of serum cytokine and chemokine parameters (CXCL10, IFN-γ, IL-6) in serum at pre-dose, and 8 h, 12 h, 24 h, 48 h post-dose and on Day 7.   + Other relevant cytokines/chemokines may be assessed if a multiplex assay can be implemented (IFN-α, MIP1α, IL-10, TNFα and/or other parameters) at pre-dose, and 8 h, 12h, 24 h, 48 h post-dose and on Day 7. * PBMC samples:   + Blood samples at pre-dose, Day 2 and Day 7 will be used to isolate PBMCs that will further be sent to the University of Tokyo for exploratory PD investigations. * mRNA markers:   + Blood sample for mRNA markers will be collected on Day 1 at pre-dose, 4 h, 8 h, 12 h, 24 h and 48 h post-dose and will be stored for subsequent exploratory analysis on cytokine and chemokine gene expression**.** | |
| **STATISTICAL METHODS:**  **Safety data:**   * **AEs:** All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA dictionary). The most up to date version that is available at the time of database build will be used and will be listed in the data management plan (DMP). The MedDRA dictionary will not be updated during the course of the study. Only treatment emergent adverse events (TEAEs), i.e., existing conditions that worsen or events that occur during the course of the study after administration of IMP, will be included within the summary tables. * **Laboratory Safety**: Biochemistry, haematology, coagulation and urinalysis parameters will be listed with any out of normal range values flagged. Laboratory test results which are out of normal range will also be presented separately along with normal reference ranges. Descriptive statistics (N, n, mean, SD, minimum, median and maximum) of absolute and change from baseline (Day -1) values at each protocol-defined time point will be tabulated. * **Vital Signs**: Vital signs parameters will be listed with any out of normal range values flagged. Descriptive statistics (N, n, mean, standard deviation (SD), minimum, median and maximum) of absolute and change from baseline (Day 1 pre-dose) values at each time point will be tabulated. * **12 Lead ECG:** 12-Lead ECG parameters will be listed with any out of normal range values flagged. Descriptive statistics (N, n, mean, SD, minimum, median and maximum) of absolute and change from baseline (Day 1 pre-dose) values at each time point will be tabulated. Additionally, the frequency (number and % of subjects) for absolute and change from baseline QTcF values will be summarised. * **Inject site examination**: Injection site reactions (redness, swelling, warmth, tenderness, pain) will be assessed during the study, according to the assessment scaled in Appendix 3, listed and summarised using frequencies (number and % of participants).   **Pharmacokinetic data**:  ***Concentration-Time data:*** Individual plasma CpG ODN D35 concentration‑time data will be listed and summarised. Individual and geometric mean concentration‑time data will also be plotted on both linear and semi‑logarithmic scales.  ***Derived PK data:*** The following PK endpoints will be derived from plasma CpG ODN D35 concentration time data following administration of CpG ODN D35 using Phoenix WinNonlin 8.0 or higher. For the final PK calculations, concentration values below the limit of quantification (BLQ) will be assigned a value of zero and the actual time of sample collection will be used.   * ***PK Variables following single SC dose administration*:** Maximum concentration (Cmax), time to Cmax (Tmax), terminal elimination half-life (t1/2), apparent first order terminal elimination rate constant (λz), area under the concentration-time curve (AUC) from time of dosing to last measurable concentration (AUClast), AUCall, AUC extrapolated to infinity (AUC0-inf), residual AUC (AUC% extrapolated). Additional parameters may be reported, as appropriate.   **Dose proportionality**:  Dose proportionality will be assessed by performing a regression analysis of the log-transformed Cmax, AUClast, and AUC0-inf values versus the log-transformed dose using the power model. For each parameter, a point estimate and 95% confidence interval (CI) will be calculated for the slope of the regression line. The Cmax, AUClast and AUC0-inf values will also be presented graphically.  **PD and Exploratory data:**  PD analysis will be detailed in statistical analysis plan (SAP) and exploratory data will be analysed and reported separately. | |
| **duration of study:** | Approximately 6 weeks for each individual (from the Screening to post study follow-up). |

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# ABBREVIATIONS USED IN THE PROTOCOL TEXT

2. ABPI Association of the British Pharmaceutical Industry
3. ADR adverse drug reaction
4. AE(s) adverse event(s)
5. ALP alkaline phosphatase
6. ALT alanine transaminase
7. API active pharmaceutical ingredient
8. aPTT activated partial thromboplastin time
9. AR adverse reaction
10. AST aspartate transaminase
11. AUC area under the concentration versus time curve
12. AUC0-inf AUC extrapolated to infinity from dosing time, based on the last measurable concentration
13. AUClast AUC from the time of dosing to the time of the last measurable concentration
14. AUCall AUC from 0 to the time of the

last observation, regardless of

whether the last concentration

is measurable or not

1. BIA BioIndustry Association
2. BLQ below the limit of quantification
3. BMI body mass index
4. bpm beat(s) per minute
5. Cmax maximum plasma concentration
6. CCRA Clinical Contract Research Association
7. CG Cytosine-Guanine
8. CK creatine kinase
9. CL cutaneous leishmaniasis
10. COVID-19 coronavirus disease 2019
11. CNS central nervous system
12. CPK creatine phosphokinase
13. CRP c-reactive protein
14. CRS cytokine release syndrome
15. CV% coefficient of variation
16. DERM dose escalation review

meeting

1. DMP Data Management Plan
2. DNDi Drugs for Neglected Diseases

initiative

1. DOA drugs of abuse
2. DoH Department of Health
3. EC European Commission
4. eCRF electronic case report form
5. ECG electrocardiogram
6. EDTA ethylenediaminetetraacetic acid
7. EU European Union
8. FIH First in Human
9. EMA European Medicine Agency
10. g gramme(s)
11. GCP Good Clinical Practice
12. GDPR General Data Protection Regulation
13. GGT gamma glutamyltransferase
14. GI gastrointestinal
15. GLP Good Laboratory Practice
16. GMP Good Manufacturing Practice
17. h hour(s)
18. HBsAg hepatitis B surface antigen
19. HED human equivalent dose
20. HCV Ab hepatitis C virus antibody
21. HIV human immunodeficiency virus
22. HR heart rate
23. HRA Health Research Authority
24. IB Investigator’s Brochure
25. ICF informed consent form
26. ICH International Council on Harmonisation
27. IMP investigational medicinal product(s)
28. IV intravenous
29. INR international normalised ratio
30. ISF Investigator site file
31. IUD intrauterine device(s)
32. IUS intrauterine system
33. LC MS/MS liquid chromatography tandem mass spectrometry
34. LLOQ lower limit of qualification
35. LSP laboratory sampling manual
36. Ltd Limited
37. MCH mean corpuscular haemoglobin

* MCHC mean corpuscular haemoglobin concentration
* MCV mean corpuscular volume
* MedDRA Medical Dictionary for Regulatory Activities
* MHRA Medicines and Healthcare products Regulatory Agency
* MRSD maximum recommended starting dose
* MTD maximum tolerated dose
* NHS National Health Service
* NK natural killer
* NOAEL no-observed-adverse-effect level
* min minute
* mmHg millimetre(s) of mercury

1. ms millisecond(s)
2. N number dosed
3. n number of observations
4. NRES National Research Ethics Committee
5. NSAID non-steroidal anti-

inflammatory drugs

* ODN Oligodeoxynucleotides

1. PBMC peripheral blood mononuclear

cells

1. PCH Prince Charles Hospital
2. PCV packed-cell volume
3. PD pharmacodynamic
4. pDCs plasmacytoid dendritic cell
5. PI principle investigator
6. PK pharmacokinetic
7. PPE personal protective equipment
8. PR interval time the electrical impulse takes to travel from the sinus node through the AV node and entering the ventricles; measured from the beginning of the P wave to the beginning of the QRS complex
9. PT prothrombin time
10. PV pharmacovigilance
11. QA quality assurance
12. QC quality control
13. QP qualified person
14. QRS complex represents ventricular depolarisation
15. QT interval the time for both ventricular depolarisation and repolarisation to occur, and therefore roughly estimates the duration of an average ventricular action potential.
16. QTc corrected QT interval
17. QTcF corrected QT interval using Fridericia’s formula
18. RBC red blood cell(s)
19. RDW red blood cell distribution width
20. REC Research Ethics Committee
21. RMP risk management plan
22. RR interval the time elapsed between two successive R waves
23. RT-PCR reverse transcription polymerase chain reaction
24. s second(s)
25. SAE serious adverse event
26. SAP statistical analysis plan
27. SAR serious adverse reaction
28. SAS statistical analysis software by SAS Institute Inc., USA
29. SC subcutaneous
30. SD standard deviation
31. SHM sample handling manual
32. SI Statutory Instrument
33. SMP safety management plan
34. SOC system organ class
35. SOP standard operating procedure(s)
36. SRC Safety Review Committee
37. SUSAR suspected unexpected serious adverse reaction
38. t½ terminal elimination half-life
39. TEAE Treatment Emergent Adverse Event
40. TSH thyroid stimulating hormone
41. TLR9 Toll-like receptor-9
42. Tmax the time to Cmax
43. TMF Trial Master File
44. TK toxicokinetic
45. UK United Kingdom
46. VL visceral leishmaniasis
47. UBC United BioSource Corporation
48. USA United States of America
49. WBC white blood cell(s)
50. ºC degrees Celsius
51. % percent

# ETHICS

## Research Ethics Committee or Institutional Review Board

This study protocol will be submitted to the Research Ethics Committee (REC) for review and provision of a favourable opinion. The favourable opinion of the REC must be obtained before commencement of any study procedures.

The favourable opinion is conditional upon the Sponsor registering the clinical trial in a publicly accessible database, within 6 weeks of the first participant recruited or following confirmation of an appropriate Health Research Authority (HRA) deferral.

All substantial protocol amendments must receive favourable opinion from the REC responsible for the study. Non-substantial amendments will not require prior favourable opinion by the REC.

If the study is stopped due to adverse events (AEs) it will not be recommenced without reference to the REC responsible for the study.

The outcome of the study (e.g., completed) will be reported to the REC responsible for the study within 90 days of completion of the last participant’s final study procedures. In the event of the study being prematurely terminated, a report will be submitted to the REC responsible for the study within 15 days.

A summary of the clinical study report will be submitted to the REC responsible for the study within 1 year of completion of the last participant’s final study procedures.

The REC will be informed that Simbec-Orion is a commercial organisation and that the study is funded by DNDi, through the support of the Japanese Global Health Innovative Technology Fund (GHIT) and DNDi core donors. The participants who take part in the clinical study will be paid for their inconvenience and have been informed that there will be no benefits gained by their participation. All potential conflicts of interest will be declared by the Investigators.

## Ethical Conduct of the Study

The Principal Investigator (PI) shall be responsible for ensuring that the clinical study is performed in accordance with the Declaration of Helsinki (Brazil 2013)[02]. It will comply with International Council on Harmonisation (ICH) Good Clinical Practice (GCP)[03] and applicable regulatory requirements.

## Participant Information and Consent

Potential participants who volunteer for participation in the study will be informed of the aims, methods, anticipated benefits and potential hazards of the study and any possible discomfort it may entail. Information will be given in both oral and written form and in the manner deemed appropriate by the Clinical Unit standard operating procedures (SOPs). Each participant will also be informed of his/her right to withdraw from the study at any time, for any reason.

A written explanation (participant information sheet) and informed consent form (ICF)will be provided, and the participant will be allowed sufficient time to consider the study information. Prior to signing the ICF, the participant will be given an opportunity to discuss any issues concerning the study with an Investigator who has suitable knowledge of the study and will have all questions answered openly and honestly.

If the participant is willing to participate in the study, the ICF will be signed and personally dated by the participant and the person taking consent. The participant will receive a copy of the ICF together with the participant information sheet. The original signed ICF will be retained with the study records at the Investigator site. In addition, the actions and completion of the consenting process will be recorded in the participant’s medical record (i.e., source document).

# INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

The study will be performed at a single site, Simbec-Orion Clinical Pharmacology Unit. The overall responsibility for the study will rest with the PI, Dr Annelize Koch. The Project Manager will act on behalf of the PI to ensure the smooth and efficient running of all aspects of the study.

## Study Personnel

**Contract Research Organisation:** Simbec-Orion, United Kingdom

* Principal Investigator: Annelize Koch
* Project Manager (Main Contact): Lan Tann
* Project Manager Deputy: Matilda Greenstreet
* Pharmacokinetics (PK): Simon Hutchings
* Statistics: Kerry Williams
* Data Management: Richard Stickley
* Laboratory Services (Safety Labs): Sara Howell
* Pharmacy: Rebecca Price-Davies

The PI will delegate study-related activities according to staff responsibilities and job descriptions. This will be documented in a study-specific delegation of responsibilities form.

**Sponsor:** DNDi, Switzerland

* Clinical Trial Manager (Main Contact): Séverine Blesson
* Clinical Trial Manager (Deputy): Bethania Blum
* Sponsor’s Medical Responsible / Responsible Physician: Byron Arana

**Monitor:** Simbec-Orion Clinical Development, United Kingdom

**Pharmacovigilance (PV):**

* DNDi PV, Switzerland
* PV provider, United BioSource Corporation (UBC), Switzerland

**Bio-analytics:** Oncodesign, France

## Indemnity Arrangements

The Sponsor and Simbec-Orion carry insurance to pay compensation for injury, accident, ill health or death caused by participation in this study without regard to proof of negligence in accordance with the insurance and compensation in the event of injury in Phase I clinical trials 2012, guidance issued by the Association of the British Pharmaceutical Industry (ABPI), the BioIndustry Association (BIA) and the Clinical Contract Research Association (CCRA) in consultation with the Department of Health (DoH) and the National Research Ethics Service (NRES)[04].

# INTRODUCTION

Leishmaniasis is generally seen as one of the most neglected tropical diseases and has strong links with poverty. It comprises a complex vector-borne disease, caused by more than 20 species of the protozoan genus Leishmania, ranging from localized skin ulcers (cutaneous leishmaniasis, CL) to lethal systemic disease (visceral leishmaniasis, VL). Leishmaniasis is endemic in 101 countries/territories, with 350 million people at risk.

Antimonials continue to be the first line treatment in most CL endemic countries despites its toxicity, difficult administration and the variable efficacy showed across different countries and species of Leishmania parasites causing CL. Miltefosine, recently registered at Food and Drug Administration (FDA, United States) for the treatment of CL in the New World (NW), is not available in most countries and its efficacy for infections due to *L. braziliensis*, the parasite responsible for the majority of CL cases in the NW, varies from 50% to 85%.

DNDi Cutaneous Leishmaniasis strategy aims to develop a short, safe and efficacious treatment for CL that can be used at any healthcare level in all disease-endemic areas.

DNDi has developed CpG ODN D35.

* Oligodeoxynucleotides (ODN) that contain unmethylated CpG dinucleotides, known as CpG-ODNs. According to their *in vitro* activities and chemical compositions, three major classes of ODN are described. Class A (also known as class D) are characterised by strong plasmacytoid dendritic cell (pDCs) and Natural Killer (NK) cell activation, resulting in high levels of *IFN-α* and *IFN-γ* production. Class B (also known as class K) are characterized by promoting a strong B cell activation. Class C have immune activities intermediate to the A- and B-classes and can activate not only B cells but also pDCs and NK cells as well.
* CpG ODN D35, is a class A CpG ODN TLR9 agonist. It is a short, single-stranded synthetic DNA molecule (20 bases in length) that contains un-methylated Cytosine-Guanine (CG) dinucleotide motifs that can be detected by TLR9 on pDCs in humans.
* CpG ODN D35 stimulates maturation and activation of pDCs and production of pro-inflammatory cytokines, such as *IFN-α* and *IFN-γ*, which are required for control of the *Leishmania* infection. CpG ODN D35 has little or no effect on B cells and does not foster the Th2 type responses associated with other classes of CpG ODN.

Given its properties, CpG ODN D35 in combination with chemotherapy has the potential to significantly improve the treatment of patients with complicated CL diseases or patients with leishmaniasis recidivans, conditions for which the standard treatment has shown to have limited efficacy (<60% cure rate) or require multiple treatment cycles. In addition, it is expected that CpG ODN D35 will reduce the required standard chemotherapy injections of antimonials and its related AE which may reduce the suffering of patients and improve compliance, particularly in children.

This study is the first to administer CpG ODN D35 to humans.

## Physical, Chemical, Pharmaceutical Properties and Formulation

The active pharmaceutical ingredient (API) is CpG ODN D35.

The investigational product will be supplied as solution for subcutaneous (SC) injection in a sterile 2R Type I vial sealed with ETFE coated bromobutyl stopper and aluminium overseal. Each vial contains 15 mg/mL of CpG ODN D35 as a 2.5 mL single-use solution. The investigational product also contains the following non-active ingredient: trehalose dihydrate (88 mg/mL) as a tonicity agent.

A placebo for CPG ODN D35 solution for injection is also provided. The placebo solution for injection is made using the same non-active excipient trehalose dihydrate (88 mg/mL) as the investigational product. The placebo has no obvious coloration whereas the CpG ODN D35 solution 15 mg/mL has a slight yellow tinge. Therefore, syringes used to administer the drug product to the subjects will be covered prior to their presentation to the Investigator to ensure blinding in clinical trials.

CpG ODN D35 solution 15 mg/mL should be stored at 2-8°C and upright in the original container.

## Nonclinical Studies

### Nonclinical Pharmacology

The pharmacological activity of an early research batch of CpG ODN D35 was investigated *in vitro* and *in vivo* and provided first evidence that CpG ODN D35 is an efficacious Class A TLR9 agonist potentially suitable for various indication[05-09].

Subsequent non-clinical development was conducted with an optimised drug candidate and focused on providing evidence on biological activity of the newly produced batch in comparison to the early research batches as well on showing proof-of-principle in relevant animal models to support the development of CpG ODN D35 for treatment of CL patients.

*In vivo* studies in cynomolgus monkeys showed that a single SC treatment with CpG ODN D35 at dose levels of ≥1 mg/kg was sufficient to induce a local and systemic (distant from injection site) pharmacological effect as shown by analysis of cytokine response (IFN-α, IFN-γ, CXCL10/IP-10 and IL-6) in peripheral blood mononuclear cells (PBMCs) and in skin collected at the injection site or skin distant from the injection site. Treatment was well tolerated up to the highest tested dose of 12 mg/kg. At 3 mg/kg a plateau in cytokine response was reached locally and systemically since an increase of the dose did not result in a further increase in the inflammatory response (cytokine response). However, in tissue samples collected from the contralateral, distant site of injection, cytokine mRNA levels increased with dose with highest response observed at the highest tested dose of 12 mg/kg. The pharmacological activity of CpG ODN D35 dose levels ≥1 mg/kg (SC) was confirmed in a combined safety and efficacy study conducted in cynomolgus monkeys. This study also provided evidence for a sustained effect of CpG ODN D35 since cytokine levels did not return to baseline in skin biopsies taken 3 weeks after the last dosing.

#### Primary Pharmacodynamics

*In vitro* studies in PBMCs of different species were conducted to support the selection of species for non-clinical testing. *In vivo* studies were subsequently initiated in cynomolgus monkey and rhesus macaques which were identified as pharmacologically relevant species. To support the use of CpG ODN D35 as adjuvant therapy to a chemotherapy in CL patients, a co-treatment with CpG ODN D35 and pentavalent antimony was included in a subset of these studies.

In addition, a proof-of-concept study in *Leishmania major* infected rhesus macaques was performed. That study included the assessment of efficacy after application of CpG ODN D35 as single treatment or after a co-treatment with pentavalent antimony.

#### Safety Pharmacology

No stand-alone safety pharmacology studies were conducted with CpG ODN D35.

Assessment of the cardiovascular and respiratory systems were included in the good laboratory practice (GLP) repeat-dose toxicity study conducted in cynomolgus monkeys (Study No. 20180061TCYP). Detailed monitoring of clinical signs was included in this study, which allows for the identification of potential effects on the central nervous system (CNS). Based on the data collected in this study, CpG ODN D35 was found to exhibit no effect on the cardiovascular, respiratory or CNS after SC dosing every two weeks up to a dose of 15.9 mg/kg for a total of 4 doses.

#### Pharmacodynamic Drug Interaction

The pharmacodynamic (PD) interaction of CpG ODN D35 with antimonial treatment was investigated in *in vitro* and *in vivo* studies [10]. The results of those studies support the use of CpG ODN D35 as adjuvant therapy for antimonial treatment of CL patients.

### Nonclinical Pharmacokinetics and Metabolism

The pharmacokinetic (PK) profile of CpG ODN D35 was evaluated in rats and monkeys after single SC or intravenous (IV) administration. A study investigating the stability of the test item in human and monkey plasma was conducted. Assessment of toxicokinetic (TK) was included in the repeat-dose toxicity studies conducted in cynomolgus monkeys. An overview of pharmacokinetic studies is provided in Table 1[11].

Table 1 Overview of Pharmacokinetic Studies and Key Results

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Study Number (GLP Status) | Model | Study Design | Dose  (mg/kg) | Key Outcome |
| **Absorption** | | | | |
| FSR-IPL-190307  (non-GLP) | OFA SD rat | s.c. / i.v.  single dose | s.c. = 159  i.v. = 318 | s.c.:  Cmax=4,703 ng/mL; Tmax=15 min  i.v.:  Cmax=107,605 ng/mL; Tmax=15 min # |
| 20180059STP  (non-GLP) | Cynomolgus monkey | s.c. / i.v.  single dose | s.c. / i.v. = 2\* | s.c.:  Cmax=124.1 ng/mL, Tmax=15 min  AUClast = 56.7 ng\*h/mL  Bioavailability=0.96-3.28%  i.v.:  Cmax=10,488.7 ng/mL; Tmax=5 min#  AUClast=3,210.4 ng/mL  t1/2=0.2 h |
| **Distribution** | | | | |
| 190154 ET  (non-GLP) | Monkey and human plasma | *In vitro* stability at 37°C | 75 ng/mL,  800 ng/mL | CpG ODN D35 is stable in human and monkey plasma at 37°C for at least 4 hours |
| Metabolism | No metabolism studies were performed. | | | |
| Excretion | No excretion studies were performed. | | | |
| Footnote: AUClast – area under the curve from first assay to the last quantifiable concentration, Cmax – maximum plasma concentration, F - Female, GLP – good laboratory practice, i.v. – intravenous, kg – kilogram, M - Male, mg – milligram, s.c. – subcutaneous, Tmax – time to maximum plasma concentration, t1/2 – terminal half-life of elimination; \* Due to a technical error, actual dose levels were 1.61, 2.49 and 2 mg/kg for s.c.; # Earliest sampling timepoint | | | | |

### Toxicology

Detailed toxicology data are presented in the Investigator’s Brochure (IB)[11]. A summary is presented below.

General toxicity of CpG ODN D35 was investigated in the cynomolgus monkey, which was identified as relevant species in *in vitro* studies studying the cytokine response after incubation with CpG ODN D35 in PBMCs from various species. No maximum tolerated dose could be identified after single dosing as a SC treatment was well tolerated up to the highest tested dose of 15.9 mg/kg.

In the pivotal GLP study in cynomolgus monkeys (Table 2), CpG ODN D35 was administered SC for 43 days every two weeks (i.e., a total of 4 doses) and a 4-week recovery period. A NOAEL of 15.9 mg/kg (highest tested dose) was determined for systemic effects (Cmax = 1230.7 and 1359.7 ng/mL and AUClast = 755.8 and 626.7 ng\*h/mL for males and females, respectively in plasma). The only adverse findings related to the test item were local reactions at the injection site including thickened skin, swelling and redness. Those findings correlated with histopathological changes as haemorrhages, SC oedema, and inflammatory cell infiltrates, occasionally accompanied by necrosis, presence of granular basophilic macrophages, extracellular subcutaneous granular material and inflammatory cysts. These signs were observed in all test item treated animals, but severity increased with dose. These signs of local intolerance might be related to the presence of the test item at the injection site, which was detected in every test item-treated group with highest tissue concentrations observed for the high dose animals. Ongoing recovery was visible at the end of the treatment-free period (4 weeks) and no test item was found at the injection site of the high dose animals at end of recovery.

Table 2 Non-Clinical GLP Repeated Toxicity Study Conducted with CpG ODN D35 and Key Outcome

| **Study No / GLP Status** | **Model / RoA** | **Study Design** | **Dose / Concentration / No of Animals per Group** | **Key Outcome** |
| --- | --- | --- | --- | --- |
| **Repeat-Dose Toxicity** | | | | |
| 20180061TCYP27  (GLP) | Cynomolgus monkey /  s.c. | Repeated dosing every 2 weeks for 43 days followed by 4 weeks of recovery | 0, 2, 6, 15.9 mg/kg  n=3M+3F/group (main study)  n=2M+2F/group (recovery; for control and high dose group only) | Treatment well tolerated systemically up to the highest tested dose. Adverse, test item-related findings restricted to local reactions at administration site (observed in all CpG ODN D35 groups). Trend towards recovery observed, although full recovery was not reached after 4 weeks of treatment-free period. NOAEL(systemic) = 15.9 mg/kg  No NOAEL for local adverse findings Cmax (Day 43)=1230.7 (M) / 1359.7 ng/mL (F); AUClast (Day 43)=755.8 (M) / 626.7 (F) ng\*h/mL |

AUC – area under curve; Cmax – maximum plasma concentration, F - Female, GLP – good laboratory practice, i.v. – intravenous(ly), M - Male, MTD – maximum tolerated dose, NOAEL – no observed adverse effect level, RoA – route of administration, s.c. – subcutaneous(ly), SD – Sprague Dawley

Based upon the toxicity program outlined above, the safety profile of CpG ODN D35 provided evidence that the investigational product is systemically well tolerated. The only adverse effects observed for CpG ODN D35 were signs of local intolerance at the injection site, when applied at a concentration of 15.9 mg/mL. However, considering the mode of action of CpG ODN D35 as a pro-inflammatory stimulant, the occurrence of signs of pro-inflammatory reactions at the injection site are expected and consistent with the known involvement of TLR9-induced signalling pathways. The presence of granular macrophages as well as subcutaneous granular basophilic material might indicate the accumulation of the test item at the injection site. This is supported by the analysis of biopsies taken from the injection site, which confirmed the presence of CpG ODN D35. Ongoing recovery was evident in the GLP toxicity study suggesting that this effect is transient. It is of further interest to note that although marked changes were present in the subcutaneous tissue with CpG ODN D35, the non-human primates did not demonstrate any clinical signs of response to local irritation and that there was no indication of any systemic toxicity related to the administration including fever.

Non-clinical testing of CpG ODN D35 did not reveal any sign for induction of flu-like symptoms. However, considering the clinical experience with ODNs and TLR9-activators, such an effect cannot be excluded for CpG ODN D35 and patients will be monitored closely after test item administration.

In addition to the assessment of general toxicity, the standard battery *in vitro* and *in vivo* genotoxicity studies was performed according to ICH and OECD guidelines. CpG ODN D35 does not have genotoxic potential in these *in vitro* and *in vivo* studies.

No dedicated studies on reproductive or developmental toxicity studies were conducted. The histopathological assessment of male and female reproductive organs, which was conducted as part of the GLP repeat-dose toxicity studies, identified no findings up to a dose of 15.9 mg/kg administered SC every two weeks for a total of 4 doses.

## Effects in Humans

Not applicable. CpG ODN D35 has never been administered and tested in humans.

Further details of the non-clinical studies and a summary of the known and potential risks and benefits to human participants of CpG ODN D35 can be found in the Investigator’s Brochure (IB)[11].

The study will be conducted in compliance with the protocol, GCP and the applicable regulatory requirement(s), as indicated in the ICH GCP E6 (R2) guidelines[03].

## Risk-Benefit Assessment

No benefit will be observed in healthy volunteers.

Several measures to minimize the risks to healthy subjects have been taken with respect to the following study design elements.

* Each cohort will follow a sentinel dose escalation schedule. 2 subjects in a sentinel group (1 subject on active drug and a subject on placebo) will be dosed at each dose level and monitored for 72 hours prior to dosing the rest of the 2 sub-groups.

No more than 3 volunteers will be dosed the same day, and 30 minutes between dosing of 2 volunteers will be ensured (except sentinels as this is known that one is receiving placebo). This is implemented to allow interruption of dosing of the rest of the sub-group in case of a severe reaction such as anaphylaxis occurring.

* For dose escalation to proceed, data from the preceding dose level must be available from a minimum of 6 evaluable subjects who have completed the planned safety assessments up to Day 4 (72h after dosing) and all planned PK assessments after dosing to ensure at least 4 subjects had received active IMP.
* The decision must be documented in writing by the SRC at the dose escalation review meeting (DERM) before the next escalated dose level is administered to volunteers.
* Potential risk of local injection site reactions: Based on pre-clinical studies (Corresponding to changes noted in Maximum Tolerated Dose (MTD) and Repeated Dose Range Finding Study in the cynomolgus monkey by the SC route), the most common effects that may be anticipated in humans are injection site reactions such as oedema and thickening of skin. In animals, this is correlated with inflammatory changes and necrosis, subcutaneous accumulation of granular material and presence of basophilic granular macrophages at the histopathology examination. These local and microscopic changes were observed at all tested dose levels, but the severity of effects was proportional to dose with highest severity observed at the highest dose of 15.9 mg/kg. Occurrence of local reactions will be monitored during the clinical trial.
* Potential Risk of systemic immune reactions: Concern has been raised in the clinical development of other classes of CpGs (developed as vaccine adjuvants) that they might activate autoreactive B cells and thus increase the risk of autoimmune disease. Baseline assessment of auto-antibodies in healthy subjects, as support for eligibility assessment, was not considered as titrations of autoantibodies did not warrant the absence of later development of the disease. Moreover, the risk of CpG ODN D35 to trigger the development of any auto-immune disease is considered very low as Class A CpGs are designed to activate plasmacytoid cells and not B cells. However, samples will be taken at baseline (Day 1 pre-dose) to analyse retrospectively (if required due to development of any auto-immune disease after study participation). Analysis could include the following biological markers (anti-dsDNA, anti-ssDNA, rheumatoid factor, anti-nuclear antibody, or others) in relation to auto-immune diseases.
* Potential Risk of cytokine release syndrome: Due to the pharmacological effect of Class A CpG ODN, as a TLR9 agonist, and the activation of Th1 cytokine production, a cytokine release syndrome (CRS) cannot be fully excluded even with a low and short-term systemic exposure linked to the subcutaneous injection, and the reassuring nonclinical data and clinical experience with CpG ODNs whatever the class. Some risk mitigation measures include: sentinel dosing, delayed dosing of the main cohort (approximately 72 hours post-sentinel dose), dividing the main cohort into 2 sub-groups 24 hours apart, limiting the number of volunteers dosed the same day, time imposed in between 2 volunteers dosing, follow up of specific markers of inflammation (such as CRP) and availability of IL-6 dosing onsite in case of need for exploration of the inflammatory response. These form a mitigation plan, alongside usual precautions associated with First-in-Human trials and will take into account the potential of prolonged pharmacodynamic effect that may result from how the synthetic molecule is designed to resist degradation (prolongation of the half-life from 5-10 minutes to 30-60 minutes of the phosphorothioate compound as compared to non-phosphorothioated natural phosphodiester DNA). A specific emergency treatment section is dedicated to this question in Section 10.6.11.
* Potential risk of anaphylaxis: Even though no clear signal was observed in nonclinical *in vivo* studies, or with other CpG ODNs nor the excipients, a risk of anaphylaxis remains possible with a SC injection of oligonucleotides. However, during this first in human (FIH) trial, the clinical site has the appropriate equipment and the site staff well trained to treat acute hypersensitivity reactions, e.g., with epinephrine/noradrenalin, antihistamines, intravenous glucocorticoids, volume expanders and/or vasopressors.
* Other safety parameters will be monitored during the study.
  + As a precaution, electrocardiogram (ECG) parameters will be carefully monitored to identify any possible prolongation of the QTc interval in healthy volunteers.
  + Slight changes in monocytes, neutrophils and eosinophil counts were observed in pre-clinical studies which will be monitored during the study.
  + No changes in blood chemistry including CRP, body weight, or temperature were observed in pre-clinical studies. These will be monitored as a standard assessment in the study with special attention to CRP in the scope of CRS occurrence monitoring.
  + No changes in coagulation parameters were observed in pre-clinical studies with CpG ODN D35. However, a low incidence of complement activation (relative to the alternative pathway) and some drug concentration-dependent prolongation of activated partial thromboplastin time (aPTT) have been noted after systemic administration of other CpG ODNs. Coagulation parameters, including aPTT, will be monitored as a standard assessment in the study, and complement evaluation will be triggered in case of fever above 38.5°C (see Section 10.6.11).

## Coronavirus Disease 2019 (COVID-19) Risk/Benefit Assessment

This study is to be conducted in healthy adult participants who are deemed to not be at higher risk of COVID-19 as per National Health Service (NHS) Guidance (<https://www.nhs.uk/conditions/coronavirus-covid-19/people-at-higher-risk/whos-at-higher-risk-from-coronavirus/>).

The safety of the participant is the primary concern; this study is to be conducted at Simbec-Orion Clinical Pharmacology Unit which is a Phase I accredited unit with extensive experience in conducting Phase I trials of similar design. Simbec-Orion prioritise the health and wellbeing of their clinical trial participants and, as such, have implemented a number of COVID-19 policies and risk mitigating actions. Prior to attendance at site, participants will be contacted to ensure they are not displaying any COVID-19 symptoms; site COVID-19 policies will be explained to them at this time. Where appropriate, Perspex screens are in place and appropriate social distancing is enforced. Where this is not possible, appropriate personal protective equipment (PPE) will be worn.

Simbec-Orion Clinical Pharmacology unit is a dedicated trial facility and, as such, staffing levels will not be affected by the potential burden presented by COVID-19 to other medical facilities. All employees present at the clinical site are aware of the COVID-19 specific working requirements and will work to the relevant ‘Working Safely’ Policy.

Medicines and Healthcare products Regulatory Agency (MHRA) Phase I Accreditation requirement No. 3 details the requirement for an agreement with a local hospital for supporting emergencies arising from the clinical trials performed by Simbec-Orion. This agreement is in place with Cwm Taf University Health Board and Prince Charles Hospital (PCH) for this purpose. Cwm Taf University Health Board and PCH have confirmed capacity to support any acute serious AEs (SAEs) during the COVID-19 pandemic (details contained within Simbec-Orion Clinical General Risk Assessment).

The investigational medicinal product (IMP) is not an immunosuppressant. There is no scientific evidence that CpG ODN D35 increases a participant’s susceptibility to COVID-19 or will exacerbate a participant’s condition should they contract COVID-19. In addition, all participants will remain in-house for the duration of the study under the medical supervision of the PI throughout.

Both the Study and Site Risk Assessments will be continually monitored and updated throughout the trial and it is currently deemed acceptable to conduct the trial without it impacting or being impacted by the COVID-19 pandemic.

In addition, the Sponsor has conducted a risk assessment in consideration of the ongoing COVID-19 vaccine deployment programme including assessment of the potential risks associated with concomitant vaccination whilst participating in this trial.

The Sponsor has determined that the outcome of the risk assessment, undertaken in conjunction with the study team, is as follows: Knowledge on the IMP is currently insufficient to assess the potential impact of IMP on the efficacy of COVID-19 vaccination or to assess the impact of COVID vaccination on IMP safety. If given concomitantly, it could possibly be difficult to discriminate if the AEs observed during the study are due to the IMP or due to COVID-19 vaccine. In addition, the study participants are young healthy volunteers, by definition without any co-morbidity, and are in consequence, in a category of subjects without any immediate risk of developing a severe form of COVID-19, and not considered the top priority for the UK vaccine campaign. Therefore, participants will not be permitted to have their COVID-19 vaccine injections whilst in the study (from 28 days prior to dosing until 28 days post-dose). Participants will be informed that if they are invited to receive a COVID-19 vaccination during the study and wish to receive the vaccine that they will be withdrawn from participation in the study. The Investigator will check that any participants who may have a pre-booked appointment for a COVID-19 vaccination, are not in breach of this requirement.

# STUDY OBJECTIVES

## Primary Objective

* To assess the safety and tolerability of a single subcutaneous dose of CpG ODN D35 in healthy male subjects.

## Secondary Objectives

* To determine PK parameters of CpG ODN D35 in plasma after single subcutaneous dose in healthy male subjects.

## PD and Exploratory Objectives

* To investigate changes from baseline levels of serum cytokine and chemokine parameters (CXCL10, IFN-γ, IL-6, optional: IFN-α, MIP1α, IL-10, TNFα and/or other parameters) after a single subcutaneous dose of CpG ODN D35.
* To investigate changes of mRNA markers after a single subcutaneous dose of CpG ODN D35, by exploratory analysis of cytokine and chemokine gene expression.
* To investigate immunological markers in PBMC isolate (for example, CXCL10, Mx1, CD80, OAS1, IRF7, IFI1 and/or other parameters).

# INVESTIGATIONAL PLAN

## Overall Study Design and Plan

The study is a Phase 1 single centre, double-blind, randomised, placebo-controlled, parallel-group, single ascending dose study of CpG ODN D35 administered subcutaneously in healthy male subjects aged between 18 and 50 years.

The study will consist of up to 4 cohorts of 8 male subjects (cohort 4 is optional). Subjects will be randomly assigned to receive a single SC dose of CpG ODN D35 (6 subjects) or placebo (2 subjects) in a sequential escalating manner.

Each cohort will follow a sentinel dose escalation schedule.

* Two (2) subjects will be dosed in each sentinel group (1 subject on active IMP and 1 subject on placebo). This design allows maintenance of the “blind”.
* The 6 remaining subjects will be randomized and split into two sub-groups with 3 subjects in each sub-group. The decision to proceed with the administration of the first sub-group of 3 subjects will be taken by the Investigator based on clinical and biological safety data after at least a 72-hour observation period of the sentinel subjects. The 3 subjects from the second sub-group will be dosed upon Investigator assessment after approximately a 24-hour observation period of the first subject of the first sub-group.
* At the end of each dose level, an interim safety report will be issued by the Investigator, for review by the SRC. No more than 3 subjects will be administered CpG ODN D35 on each day. A minimum interval of 30 minutes between dosing of any 2 subjects will be ensured (not required for sentinels).

Each cohort will follow a Screening Period, Treatment Period and Post Study Follow-up visit (approximately 6 weeks for each individual, from the screening to post study follow-up). The study sequence is presented in Figure 1.

**Figure 1: Study Sequence**

|  |
| --- |
| Day -28 to Day -2  Screening |
| ˅ |
| Day -1  Admission  Baseline Biology |
| ˅ |
| Day 1  Randomisation  CpG ODN D35 or placebo  Dose  Subjects under close medical supervision for at least 12-14 hours post-dose |
| ˅ |
| Day 1-3  Inpatient  Monitoring |
| ˅ |
| Day 4  Subject discharge |
| ˅ |
| Day 7 (Re-visit)  Day 14 (Follow-Up Visit) |

The clinical phase is anticipated to take place between May and August 2021. The end of trial is defined as last participant last visit.

The study will take place in the Clinical Unit of Simbec-Orion Clinical Pharmacology (Clinical Unit) under full medical and nursing supervision.

A schedule of all study assessments is provided in Table 5.

## Dose Escalation Procedures and Stopping Criteria

### Dose Escalation Procedures

For dose escalation to proceed, data from the preceding dose level must be available from a minimum of 6 evaluable subjects who have completed the planned safety assessments up to Day 4 (72h after dosing) and all planned PK assessments after dosing to ensure at least 4 subjects had received active IMP.

All available safety data, including follow-up data from lower/previous dose cohorts, will be reviewed at the time of dose-escalation.

Subjects will be deemed as evaluable for dose escalation purposes if they have received the planned study dose and had sufficient plasma samples collected to estimate, if possible Cmax and AUClast irrespective of whether they have received active or placebo treatment.

Planned doses may be modified following a review of emerging data. Dose escalation will be dependent upon the accrual of acceptable safety (and PK) data. If it is not appropriate to escalate the dose according to the proposed dose escalation schedule, then the same dose (only where no dose escalation stopping rules have been met), an intermediate dose or a lower dose may be given following discussion between the Sponsor and the PI (or deputy). The timing, type and number of safety, PK and PD assessments may be modified. The number and/ or volume of blood samples per assessment may be increased as long as the total volume of blood drawn for a participant does not exceed 10% of the pre-specified total blood volume or surpass 500 mL (except when extra blood samples need to be taken for safety reasons).

There will be a Telephone Conference at a pre-appointed time to involve the Simbec-Orion Project Manager and Principal Investigator (or deputy) and the Sponsor’s representative(s), including the Sponsor's Responsible Physician. After discussion of all the data, the decision will be made whether to dose escalate and a written document (dose escalation approval form) signed by the PI (or deputy) and Sponsor will be produced ratifying that decision. Full minutes, to be agreed by all parties, will be produced for each discussion regarding dose escalation and filed in the Investigator site file (ISF). A copy of the signed dose escalation approval form will be provided to the Simbec-Orion Pharmacist and this will allow the IMP to be assembled for the next dose level.

### Trial Stopping Criteria

Dosing will be temporarily stopped (via an initial email notification to REC/MHRA and then a temporary halt substantial amendment) pending evaluation of all available data if any of the following criteria are fulfilled:

* A serious adverse reaction (SAR) (i.e., a serious adverse event (SAE) considered at least possibly related to CpG ODN D35) in one subject, or
* ‘Severe’ non‐serious adverse reactions (AR) (i.e., severe non‐serious adverse events considered as, at least, possibly related to CpG ODN D35) in two subjects in the same cohort, independent of within or not within the same system‐organ class.
* Any other event deemed to pose an unacceptable risk to individuals by the PI.

If, after an internal safety review, it is appropriate to restart the trial, a substantial amendment will be submitted to the MHRA and Ethics Committee within a period of 2 years from the temporary halt date, providing the justification for the restart. The trial will not restart until the amendment has been approved by MHRA and Ethics Committee.

### Dose Escalation Stopping Criteria

A dose level will not be repeated, or increased, if the results of safety tests give the Sponsor or Investigator cause for concern, or if:

* Any of the trial stopping criteria are reached (see above).
* There are clinical indications that could suggest a moderate/severe documented CRS \*

\* CRS can present from mild flu-like syndrome, fever, fatigue, headache, cough, tachypnoea, rash, arthralgia, myalgia, hypotension or high fever and can progress to an uncontrolled systemic inflammatory response with vasopressor-requiring circulatory shock, severe dizziness and confusion, vascular leakage, disseminated intravascular coagulation, multi-organ system failure, acute respiratory distress syndrome, renal failure or signs of cardiac dysfunction with reduced ejection fraction on ultrasound, vascular leakage with peripheral and/or pulmonary oedema. Laboratory abnormalities that are common in patients with CRS include cytopenias, elevated creatinine and liver enzymes, deranged coagulation parameters, and a high c-reactive protein (CRP).

* + Two of the subjects report severe flu-like symptoms, high fever, fatigue, headache, cough, tachypnoea, rash, arthralgia, myalgia or hypotension, and considered at least possibly related to IMP.
  + Or if one subject report circulatory shock, severe dizziness and confusion, vascular leakage, disseminated intravascular coagulation, multi-organ system failure or acute respiratory distress syndrome and considered at least possibly related to IMP.
  + Or if 2 subjects have laboratory abnormalities such as cytopenias, elevated creatinine and liver enzymes, altered coagulation parameters, or elevated CRP levels: AST or ALT > 3ULN (without CPK increase) or ALP > 1.5 ULN or total bilirubin > 1.5 ULN, serum creatinine > 1.5 ULN, platelet count below 100 10^9/L, lymphocyte count below 0.5 10^9/L, CRP >20 mg/L, and considered related to the IMP and confirmed 24 to 48 hours later.
* If the dose in a single subject is anticipated to exceed an AUClast of ≥755 ng\*h/mL. These limits are based on the exposure observed at the 15.9 mg/kg in cynomolgus monkeys (systemic NOAEL: AUClast of 755.8 ng\*h/mL in males).
* If the dose in a single subject is anticipated to exceed a Cmax of ≥ 1230 ng/mL. These limits are based on the exposure observed at the 15.9 mg/kg in cynomolgus monkeys (systemic NOAEL: Cmax of 1230.7 ng/mL in males).
* If 2 subjects have grade 3 or above injection site reactions which are related to IMP injects,
* The Investigator considers the dose level to be not well tolerated.

If any of dose escalation stopping criteria are met, dosing will be halted and the SRC will be convened to explore whether it is reasonable to continue the study. All SAEs, if any, will be reported to the MHRA as per guidance.

Individual participants may also be withdrawn for any of the reasons outlined in Section 10.5.5.

If any of the above criteria are fulfilled, dose escalation will only proceed once an appropriate substantial amendment has received regulatory approval from the MHRA and ethical approval from the ethics committee associated with the study.

Planned doses may be modified following a review of emerging data. Proposed maximum clinical dose of 180 mg will not be exceeded in this study. If the Sponsor determines that it is appropriate and necessary to exceed the current planned maximum dose of 180 mg, this will not be implemented until a substantial amendment has been submitted and receives approval from both the REC and MHRA.

Dose escalation will be dependent upon the accrual of acceptable safety (and PK) data. If it is not appropriate to escalate the dose according to the proposed dose escalation schedule, then the same dose (only where no dose escalation stopping rules have been met), an intermediate dose or a lower dose may be given following discussion between the Sponsor and the PI (or deputy). The timing, type and number of safety, PK and PD assessments may be modified. The number and/ or volume of blood samples per assessment may be increased as long as the total volume of blood drawn for a participant does not exceed 10% of the pre-specified total blood volume or surpass 500 mL (except when extra blood samples need to be taken for safety reasons).

## Discussion of Study Design, including the Choice of Control Groups

The design of the study has taken FIH study requirements into consideration with a “dose leader” design for built-in safety.

Each cohort will follow a sentinel dose escalation schedule. Two (2) subjects will be dosed in each sentinel group (1 subject on active IMP and 1 subject on placebo). The 6 remaining subjects will be randomized and split into two sub-groups with 3 subjects in each subgroup. The decision to proceed with the administration of the first sub-group subjects will be taken by the Investigator based on clinical and biological safety data after at least a 72-hour observation period of the sentinel subjects. The 3 subjects from the second sub-group will be dosed upon Investigator assessment after approximately 24-hour observation period of the first subject of the first sub-group.

At the end of each dose level, an interim safety report will be issued by the Investigator, for review by the SRC. No more than 3 subjects will be administered CpG ODN D35 each day. A minimum interval of 30 minutes between dosing of any 2 subjects will be ensured (not required for sentinels).

The intended route of administration is SC. The IV route was used in the micronucleus rat study only to ensure a maximum exposure of the bone marrow. This of course does not relate to CpG ODN D35 mechanism of action against Leishmania infection.

Follow-up period of 2 week is considered appropriate based on the estimated relatively short half-life of the IMP.

## Selection of Study Population

Up to thirty-two (32) participants (4 cohorts of 8 healthy male subjects, cohort 4 is optional) will be required to complete the study.

The study is to be conducted in healthy participants; therefore, participants are not expected to derive any therapeutic benefit from taking part in the study. A healthy participant population with carefully considered inclusion/exclusion criteria will avoid the potential for interaction of CpG ODN D35 with any underlying disease state or concomitant medication that it may be necessary for patients to take, while ensuring that participants are fit and well enough for participation in the study.

CpG ODN D35 has not been previously administered to humans; therefore, its effects in humans are as yet unknown.

The following eligibility criteria are designed to select participants for whom protocol treatment and procedures are considered appropriate. All relevant medical and non-medical conditions should be taken into consideration when deciding whether this protocol is suitable for a particular participant.

Deviations from inclusion and exclusion criteria are not allowed as deviations have the potential to impact the scientific integrity of the study, regulatory acceptability or participant safety as such deviations constitute a deliberate breach of Regulation 29 of SI 2004/1031. Therefore, adherence to the criteria as specified in the protocol is essential.

### Inclusion Criteria

**To be confirmed at screening:**

1. Male healthy subjects 18 to 50 years old at the time of obtaining the informed consent.
2. Body weight ≥60 kg to ≤ 90 kg, BMI 18 to 30.1 kg/m2. BMI = body weight (kg) / [height (m)]2
3. Provision of written informed consent to participate as shown by a signature on the participant information sheet and consent form, after reading the information sheet and consent form, and after having the opportunity to discuss the trial with the Investigator or his/her delegate.
4. Normal blood pressure: Systolic blood pressure between ≥100 and ≤140 mmHg, Diastolic blood pressure ≤ 90 mmHg, measured after 10 min rest in supine position at Screening, admission, and pre-dose.
5. A resting heart rate (HR) between ≥45 and ≤90 bpm measured after 10 min rest in supine position at Screening, admission, and pre-dose
6. ECG recording without clinically significant abnormality, including a QTcF measure of ≤ 450 msec.
7. Male participant (and partner of childbearing potential) willing to use a highly effective method of contraception (see Section 10.5.1), if applicable (unless anatomically sterile or where abstaining from sexual intercourse is in line with the preferred and usual lifestyle of the participant) from first dose until 3 months after last dose of IMP.
8. No clinically significant history of previous allergy / sensitivity to CpG ODN D35 or any of the excipients contained within the IMP(s).
9. No clinically significant abnormal test results for serum biochemistry, haematology and/or urine analyses within 28 days before the first dose administration of the IMP.
10. Participant with a negative urinary drugs of abuse (DOA) screen (including alcohol/cotinine) test results, determined within 28 days before the first dose administration of the IMP (N.B.: A positive test result may be repeated at the Investigator’s discretion).
11. Participant must be available to complete the study (including all follow-up visits).
12. Participant must satisfy an Investigator about his fitness to participate in the study.

**To be re-confirmed on Day -1 / prior to first dose administration:**

1. Participant continues to meet all Screening inclusion criteria.
2. Participant with a negative urinary drugs of abuse screen (including alcohol/cotinine) prior to first dose administration.

### Exclusion Criteria

**To be confirmed at Screening:**

1. Behavioural, cognitive, or psychiatric disease that in the opinion of the Investigator affects the ability of the participant to understand and cooperate with the study protocol.
2. History of clinically significant cardiovascular, renal, hepatic, neurological (especially seizures), immunological, psychiatric, myopathies, bleeding tendency, respiratory and particularly GI disease, especially peptic ulceration and chronic gastritis, GI bleeding, ulcerative colitis, Crohn’s Disease or Irritable Bowel Syndrome, as judged by the Investigator.
3. Individual or family history of pre-existing autoimmune or antibody-mediated diseases including (but not limited to): systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, Sjögren's syndrome, type 1 diabetes mellitus, auto-immune thyroiditis, Basedow syndrome, autoimmune thrombocytopenia; or proteinuria (greater than trace protein on urine dipstick testing).
4. History of allergy, hay fever, intolerance or photosensitivity to any drug or have a history of serious allergy, asthma, allergic skin rash or sensitivity to any drug.
5. Subjects who are taking, or have taken, any prescribed or over-the-counter drug (including non-steroidal anti-inflammatory drugs (NSAID)) in the 28 days or 5 half-lives (whichever is longer) before IMP administration. Administration of up to 3 g of paracetamol per day within 7 days of IMP administration is allowed.
6. Subjects who have received any prophylactic vaccine (including COVID-19 vaccine) or immunization within the last 28 days or use of corticosteroids or immunosuppressive drugs within 28 days of IMP administration.
7. Subjects with febrile illness or infectious illness within 2 weeks of IMP administration.
8. Subjects with positive hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (HCV Ab) and or human immunodeficiency virus (HIV) tests results at Screening.
9. Positive RT-PCR COVID19 test at admission.
10. Donation or loss of greater than 500 mL of blood within the previous 3 months prior to IMP administration.
11. Major surgery within 12 weeks prior to Screening.
12. Subjects who are known or suspected alcohol abusers (more than 14 units of alcohol per week, one unit = 8 g or about 10 mL of pure alcohol). Positive alcohol test at Screening or admission.
13. Demonstrating excess in caffeine/xanthine consumption (more than 6 cups of coffee or equivalent a day).
14. History of use of drugs of abuse in the past 2 years.
15. Subjects who do not have suitable veins for multiple venepunctures/cannulation.
16. Subjects who have any clinical condition or prior therapy which, in the opinion of the Investigator, could jeopardize the safety or rights of a volunteer participating in the trial or would render them unable to comply with the protocol.
17. Participation in a non-marketed drug clinical study within 3 months or five half-lives (whichever is longer) or a marketed drug clinical study within 30 days or five half-lives (whichever is longer) before the first dose of IMP (washout period between studies is defined as the period of time elapsed between the last dose of the previous study and the first dose of the next study).
18. Subjects who are study site employees, or immediate family members of a study site or sponsor employee.
19. Inability to communicate well with the Investigators (i.e., language problem, poor mental development or impaired cerebral function).
20. Users of nicotine products i.e., current smokers or ex-smokers who have smoked within the 6 months prior to Screening or users of cigarette replacements (i.e., e-cigarettes, nicotine patches or gums).

**To be re-confirmed at Day -1 / prior to fist dose administration:**

1. Development of any exclusion criteria since the Screening visit.

## Additional Advice and Restrictions for Study Population

### Contraception

To prevent pregnancy, male participant (and partner of childbearing potential) must be willing to use a highly effective method of contraception, if applicable (unless anatomically sterile or where abstaining from sexual intercourse is in line with the preferred and usual lifestyle of the participant) from first dose until 3 months after last dose of IMP.

Highly effective methods of contraception include:

* Combined (oestrogen and progestogen containing) hormonal contraception (oral, intravaginal and transdermal) associated with inhibition of ovulation,
* Progestogen-only hormonal contraception (oral, injectable and implantable) associated with inhibition of ovulation,
* Intrauterine device (IUD),
* Intrauterine hormone-releasing system (IUS),
* Bilateral tubal occlusion,
* Vasectomised partner (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate).

To prevent exposure of any partner (male or female) during non-vaginal intercourse to the semen from a male participant who has been exposed to the IMP, the following contraception must be used:

* Condom.

The chosen contraception method(s) must be followed from the first dose until at least 3 months after receiving IMP.

Male participants must use a condom when engaging in sexual intercourse with a female who is pregnant or breastfeeding during the study.

### Sperm Donation

Participants must not donate sperm from the first dose and for at least 3 months after receiving IMP.

### Diet and Fluid Restrictions

#### Mealtimes/Fasts

At least 8 hours fasting is required prior to dosing on Day 1. All meals are served after completion of all examinations planned at a given timepoint.

Breakfast will be served: approximately 30 min post-dose.

Lunch will be served: approximately 4 h post-dose.

Dinner will be served: approximately 8 h post-dose.

Snack will be served: approximately 12 h post-dose.

On all non-dosing study days, whilst resident in the Clinical Unit, meals will be served at standard times.

Participants will choose meals from a standard menu while resident at the Clinical Unit.

Subjects are required to fast for at least 8 h prior to laboratory sample collection for the following tests: Blood glucose (serum).

#### Fluid Intake

No fluid restrictions.

Only decaffeinated tea and coffee as well as squash/cordial are available while resident at the units.

#### Alcohol Intake

Subjects must abstain from alcohol during the 24 h prior to Screening and the 48 h prior to admission until discharge from the study. Subject must have a negative alcohol screen at admission.

From Day -7 to Day -3, the consumption of alcohol will be limited to a maximum of 2 units per day. Any deviation outside this alcohol intake restriction will be assessed on a case-by-case basis at Investigator’s discretion (provided the subject’s alcohol intake will not impact in the safety aspects and objectives of the study).

#### Caffeine

Food or drink containing caffeine or other xanthines, including coffee, tea, cola, energy drinks or chocolates will be avoided completely 2 days prior to dosing (24 hours prior to admission on Day -1) until discharge from the study.

#### Poppy and Sesame Seeds

Participants will be advised that they must not eat food containing poppy and/or sesame seeds for 3 days before each visit to the Clinical Unit, as consumption of poppy and/or sesame seeds can lead to a positive opiate result in the DOA test.

### Other Life-Style Restrictions

#### Strenuous Exercise

Strenuous exercise must be avoided completely from 3 days before dosing of IMP until the final study visit.

#### Blood Donation

Participants will be advised that they should not donate blood for at least 3 months after the final study visit.

#### Phototoxicity

Phototoxicity has not been assessed in pre-clinical studies. As a precaution, it is recommended that subjects should not exposed themselves to sun for up to follow-up visit following dose.

### Removal of Participants from Therapy or Assessment

Each participant will be informed of their right to withdraw from the study at any time and for any reason.

An Investigator will withdraw a participant from the study at any time for any of the following reasons:

* If a participant experiences a serious or intolerable AE, that prevents them from continuing.
* If a participant incurs a significant protocol violation which impacts on their safety or the scientific integrity of the study (this will be discussed on a case‑by‑case basis with the Sponsor).
* At the request of the Sponsor.
* If it is considered that the participant’s health is compromised by remaining in the study or the participant is not sufficiently cooperative.
* If a participant is lost to follow-up.
* If a participant meets any of the stopping criteria specified in Section 10.2*.*

The reasons for any participant withdrawal will be recorded on the study completion form of the electronic case report form (eCRF).

If a participant is withdrawn or chooses to withdraw from the study for any reason, every possible effort will be made to perform the evaluations described for the post study follow-up (see Table 5). The safety data collected from withdrawn participants will be included in the study report.

In the event of any abnormalities considered to be clinically significant, participants will be followed-up with appropriate medical management until values are considered to be clinically acceptable. Referral or collaborative care will be organised if considered necessary.

Up to thirty-two (32) participants are required to complete the study. Participants who withdraw from the study before receiving any IMP will be replaced. Participants who are withdrawn from the study due to significant drug-related AEs will not be replaced. Replacement of all other participants withdrawn from the study after receiving IMP will be decided on a case‑by‑case basis by the PI (or deputy) and Sponsor.

## Investigational Medicinal Product

### Identity

The investigational product (active and placebo) will be supplied as a solution for SC injection in a sterile 2R Type I vial sealed with ETFE coated bromobutyl stopper and aluminium overseal. Each vial of active contains 15 mg/mL of CpG ODN D35 as a 2.5 mL single-use solution. The investigational product also contains the following non-active ingredient: trehalose dihydrate (88 mg/mL) as a tonicity agent.

A placebo for CpG ODN D35 solution for injection is also provided. The placebo solution for injection is made using the same non-active excipient trehalose dihydrate (88 mg/mL) as the investigational product.

The identity of each IMP is detailed in Table 3.

Table 3 Identity of Investigational Medicinal Products

|  |  |  |  |
| --- | --- | --- | --- |
| **IMP Name** | **Strength** | **Presentation/Form** | **Route** |
| CpG ODN D35 | 15 mg/mL | Solution for injection | SC injection |
| Placebo for CpG ODN D35 | N/A | Solution for injection | SC injection |

The placebo has no obvious coloration whereas the CpG ODN D35 solution 15 mg/mL has a slight yellow tinge. Therefore, syringes used to administer the drug product to the subjects will be covered prior to their presentation to the Investigator to ensure blinding in clinical trials.

The planned doses are presented below:

* Cohort 1: SC injection of 7.5 mg (0.5 mL, approximately 0.1 mg/kg) of CpG ODN D35 (6 subjects) or placebo (2 subjects)
* Cohort 2: SC injection of 22.5 mg (1.5 mL, approximately 0.3 mg/kg) of CpG ODN D35 (6 subjects) or placebo (2 subjects)
* Cohort 3: SC injection of 67.5 mg (4.5 mL, approximately 1.0 mg/kg) of CpG ODN D35 (6 subjects) or placebo (2 subjects)
* Cohort 4 (optional): SC injection of 180 mg (12 mL, approximately 3.0 mg/kg) of CpG ODN D35 (6 subjects) or placebo (2 subjects)

This progression of doses is provided for information. A maximum increase of 3-fold in between cohorts is envisaged based on the dose/concentration-response curves observed in in vitro and in vivo models. This will be decided by the SRC at the end of each cohort.

### Receipt and Storage

The IMP (CpG ODN D35 and placebo) will be supplied by the Sponsor.

The Sponsor must notify the PI, or the Project Manager, prior to dispatch of IMP supplies, and of the anticipated date of their arrival. IMP should arrive at the study site at least 7 days before the first dosing day. The Sponsor shall address all supplies to:

The Production Manager

The Pharmacy  
Simbec-Orion   
Merthyr Tydfil Industrial Park  
Merthyr Tydfil CF48 4DR

Upon receipt, supplies will be dealt with as per Simbec-Orion SOP SR-IMP 053. Temperature monitors included with shipments will be downloaded.

The IMPs will be stored under quarantine in a segregated, study-specific area, in a secure, temperature-controlled pharmacy.

* Formal stability studies with the investigational product are ongoing under long term (2-8°C) and accelerated (25°C/60% RH) conditions. Results to date indicate that CpG ODN D35 solution 15 mg/mL is stable when stored upright in 2R vial at 2-8°C.

The shipping documentation and bulk product qualified person (QP) certification will be reviewed. The supplies will subsequently be removed from quarantine and approved for use.

### Manufacture and Release

Subject-specific syringes of IMP will be prepared by suitably trained Simbec-Orion staff according to the Simbec-Orion SOP SR-IMP 015.

The IMP will be labelled as specified in Annex 13 (manufacture of IMPs) of the European Commission (EC) guide to Good Manufacturing Practice (GMP)[12].

The finished IMP will be certified by Simbec-Orion’s QP according to the Simbec-Orion SOP SR-IMP 030.

### Administration

A single SC injection of IMP or placebo will be administered in the morning on Day 1, according to the randomisation, in semi-supine position after at least 8 hours fasting.

The average fill volume in a vial is 2.5 mL and the injection volume will be 2.3 mL or less per SC injection site. For doses requiring a volume above the 2.3 mL, or in case the PI considers more appropriate to decrease the volume per injection site due to feasibility constraints, additional injections in different locations can be used for administration. The location of injection is important for subcutaneous injections. The drug needs to be injected into the fatty tissue just below the skin. Some areas of the body have a more easily accessible layer of tissue, where a needle injected under the skin will not hit muscle, bone, or blood vessels. The most common injection sites are:

1. Abdomen: above or under the level of the belly button, about two inches away from the navel.
2. Arm: back or side of the upper arm.
3. Thigh: front of the thigh.

The dosing will potentially necessitate several successive injections in different injection sites, given sequentially as soon as possible within a maximum of 3 minutes on Day 1.

IMP administration will be documented in the eCRF.

There will be at least 7 days between dosing of 2 different dose levels.

### Return/Destruction

All used IMP containers and unused IMP will be held under quarantine pending return/destruction*.* Drug accountability will be performed according to Simbec-Orion SOP SR-IMP 043 (Dispensing and Issue of IMP and NIMP) and SOP SR-IMP 044 (final reconciliation and destruction).

The Sponsor must provide approval for return/destruction of all remaining IMP within 8 weeks study completion. After this period, a charge for storage will be incurred.

All returns will be arranged at the earliest available delivery date. For IMP destruction, the Sponsor will receive the Certificate of Destruction 4 to 6 weeks from the date of removal from site.

### Method of Assigning Participants to Treatment Groups

Participants will be allocated to treatment groups according to a randomisation code produced by Simbec-Orion using the PROC PLAN procedure of SAS® (the most up to date version will be used and this will be documented in the statistical analysis plan (SAP)). The randomisation code will include 2 dose-leaders (1 active:1 placebo) in each cohort.

Participants will be numbered sequentially from 001 (i.e., 001, 002 etc.). Replacement participants will be assigned the same randomisation as the participant they are replacing, however, 100 will be added to the number (i.e., 101 would replace 001 etc.).

### Selection of Doses in the Study

#### Starting Dose

The starting dose of CpG ODN D35 for the FIH study is selected based on FDA (2005) and European Medicines Agency (2017) guidelines using nonclinical data.

The NOAEL-based maximum recommended starting dose (MRSD) according to the FDA was calculated using the NOAEL from Cynomolgus monkey, which was identified as the relevant species in in vitro studies studying the cytokine response after incubation with CpG ODN D35 in PBMCs from various species. The NOAEL, as derived from the repeat-dose 43 day study GLP in Cynomolgus monkey, was 15.9 mg/kg. The human equivalent dose (HED) was calculated as 15.9 mg/kg / 3.1 = 5.13 mg/kg. Applying a standard safety factor of 10 (per FDA guidance), the NOAEL based MRSD would be 5.13/10 = 0.513 mg/kg.

With respect to pharmacological activity, *in vivo* assessment of the biological activity of CpG ODN D35 in Cynomolgus monkey identified a single SC dose of 1 mg/kg as a pharmacologically active dose. The HED was calculated as 1 mg/mg / 3.1 = 0.32 mg/kg.

Taking a conservative approach, a human starting dose of 0.1 mg/kg by SC administration is proposed. A summary of safety margins to nonclinical safety and efficacy data is provided in Table 4.

Table 4 Overview of Safety Margins to Planned Clinical Starting Dose

|  |  |  |
| --- | --- | --- |
| **Endpoint** | **HED** | **Safety Margin to Planned Clinical dose of 0.1 mg/kg** |
| Systemic Toxicity  Non-human primate  (NOAEL = 15.9 mg/kg) | 5.1 mg/kg1 | 51 |
| Efficacy *in vivo*  Non-human primate (1 mg/kg) | 0.32 mg/kg1 | 3.2 |
| Biological Activity *in vitro*  Human PBMCs  (6.7 µg/mL) | 0.3 mg/kg2 | 3 |

1. HED was calculated by applying a conversion factor of 3.1 to the animal dose as recommended by FDA Guidance.

2. Efficacious concentration in human PBMCs = 6.7 µg/mL à for 60 kg patient (2778 mL plasma) = 18.6 mg/patient (0.3 mg/kg)   
HED – human equivalent dose, NOAEL – no observed adverse effect level PBMC – peripheral blood mononuclear cells

#### Dose Range to be Investigated

Doses to be assessed in this First-in-Human study were calculated by allometry, as PK-PD correlation is of limited predictive value with CpG ODNs. Exposure prediction potentially associated with the starting dose in humans was not considered relevant to predict the possible biological effects of CpG ODN D35.

Oligodeoxynucleotides containing immunostimulatory CpG motifs (CpG ODN) act as potent Th1-like immune enhancers through activation of TLR9 and their potential role as vaccine adjuvants and as therapeutic immune modulators have been described in several diseases, including e.g. hepatitis B, hepatitis C, or influenza. Their pharmacodynamic effects are observed at relatively low doses and pharmacokinetics parameters do not reflect well their immunomodulatory potency. In particular, the half-life of these compounds in blood is short.

However, after first dose, predictions will be conducted based on observations made from previous dose. No dose level that is predicted to exceed either the Cmax or AUClast seen at the NOAEL in the nonclinical studies will be administered in this study, without prior approval (via substantial amendment) from REC and MHRA. During the study, PK data from the previous cohort/s, along with safety and tolerability information, will be used to aid review of planned dose levels for the subsequent cohorts.

#### Dose Escalation Increments

The selected human starting dose of 0.1 mg/kg is 51-fold lower (scaling based on body surface area) than the systemic NOAEL of 15.9 mg/kg identified in cynomolgus monkeys. No signs of systemic toxicity were observed that would preclude the use of CpG ODN D35 in humans at the intended initial dose of 0.1 mg/kg.

The proposed maximum dose escalation increment for all cohorts (with the exception of cohort 1) is up to a 3-fold increase.

Dose levels investigated may vary from those planned but will not exceed the fold-increases outlined above. The Sponsor and the PI may decide not to proceed with further dose cohorts, to administer a lower or intermediate dose, to repeat a dose in subsequent cohorts or to proceed to a higher dose if at all. It is noted that escalation/continuation to the next dose level will not occur if the exposure observed in any individual participant exceeds those levels seen at the NOAEL in the cynomolgus monkey (Cmax ≥ 1230 ng/mL and/or AUClast ≥ 755 h\*ng/mL), or if they are anticipated to do so at the next dose level based on emerging PK data.

At each dose level, all subjects will receive the same dose, i.e., dose is not related to body weight. However, the maximum clinical dose will be 180 mg (corresponding to 3.0 mg/kg for a 60 kg body weight subject, 2.0 mg/kg for a 90 kg body weight subject) which is 1.7-fold below the NOAEL of 15.9 mg/kg (based on body surface scaling) determined in the GLP pivotal study and is therefore considered sufficiently covered.

Four cohorts are envisioned. Cohort 4 is optional. As indicated, starting dose, established at 0.1 mg/kg is considered to be safe. Subjects in subsequent cohorts will receive approximately 0.3, 1.0 and 3 mg/kg.

In addition, it is noted that the proposed maximum clinical dose of 180 mg will not be exceeded in this study. If the Sponsor determines that it is appropriate and necessary to exceed the current planned maximum dose of 180 mg, this will not be implemented until a substantial amendment has been submitted and receives approval from both the REC and MHRA.

### Timing of Dose for Each Participant

Doses will be administered in the morning on Day 1.

Each cohort will follow a sentinel dose escalation schedule.

### Blinding

A designated individual from the IMP Management Department at Simbec-Orion will generate the randomisation code under the guidance of a statistician. All other site personnel (except PK analysts) and Sponsor personnel involved in the study will be blinded with regards to the IMP being administered. The Pharmacist (or designee) responsible for the preparation of participant doses and emergency code break envelopes will not be blinded and a copy of the original randomisation code will be issued to the pharmacist (or designee) for this purpose. PK analysts are not blinded so as to ensure PK concentration data may be received even in the event of a PD / subject withdrawal of unblinding potential.

The Bioanalytical Scientist (at Oncodesign) will be provided with a copy of the randomisation code for the purposes of analysing samples. The Bioanalytical Scientist will provide the drug concentration data for interim analysis and dose escalation data review in a re-coded participant number format, presented by dose level, in order to maintain the blind of study personnel.

**Participant doses:** Once the randomisation code has been authorised as per Simbec-Orion SOPs, each participant dose will be packaged and labelled for individual participants by designated individuals from the IMP Management Department at Simbec-Orion on behalf of the Sponsor.

**Code break envelopes:** Once the randomisation code has been authorised as per Simbec-Orion SOPs, the Pharmacist (or designee) will produce individual sealed code-break envelopes that contain the treatment allocation(s) for each participant. The envelopes will be stored in a restricted access area. A set of code break envelopes will also be provided to the Sponsor pharmacovigilance (PV).

*Emergency unblinding:* Where the site requires emergency access to an individual participant code because the Investigator believes that knowledge of the IMPs received by a participant is essential for appropriate treatment of an AE, Simbec-Orion will break the blind via the code break envelopes, if necessary, without prior consultation with the Sponsor. In such an event, the Sponsor will be notified as soon as possible via email and the treatment allocation for this subject will be unblinded for the site and the sponsor.

If the blind needs to be broken for an individual participant, the date and reason will be recorded in the participant’s eCRF. If the code is broken for any individual participant, the participant will be withdrawn from the study and the procedures accompanying withdrawal performed. If the code is broken without justification, this will be deemed a serious protocol deviation.

### Prior and Concomitant Therapy

**Prior Medication:** Prescription or non-prescription drugs, including, vitamins, herbal and dietary supplements should not be taken within 28 days (or 5 half-lives (whichever is longer)) prior to the first dose of IMP.

**Concomitant Medication:** All medications including over-the-counter products shall be avoided during the study except for the treatment of AE. COVID-19 vaccine is not allowed from 28 days prior to dosing until 28 days post-dose.

If intake of any prior or concomitant medication is necessary during the study, the daily dosage, duration and reasons for administration will be recorded on the participant’s eCRF.

### Emergency Treatment

The Investigator site, where this FIH trial is to be carried out, will be equipped with the relevant armamentarium to address any potential medical emergency. Site clinical staff will be trained to immediate life support standards and physicians to advanced life support standards and experienced in handling medical emergencies.

The clinical site will be close to and have access to an intensive/critical care unit (ICU) who can support as needed. A documented procedure for the transfer to the local hospital is available at the clinical site. The local hospital and ICU will be aware of the activity of the clinical site (days of administration and number of subjects) and the potential risk of CRS with the CpG ODN D35. The ICU will be familiar with the current international guidance for the treatment of CRS and have access to drugs acting against severe/life-threatening CRS such as tocilizumab +/- corticosteroids.

In case of symptoms and particularly fever (> 38°C) with or without constitutional symptoms evoking a CRS, the subject will be placed on continuous monitoring of heart rate, blood pressure, ECG, oral temperature, and pulse oximetry.

* A cannula for saline intravenous perfusion will be inserted in a peripheric vein (forearm).
* Antipyretics and analgesics as acetaminophen/paracetamol should be used at recommended doses and documented.
* The use of NSAID is not recommended.
* Antihistamines could be used in case of skin rash.
* For temperatures reported/recorded above 38.5°C, the following blood specimens will be collected as soon as possible to monitor the immune activation: interleukin-6 and complement samples (CH50, C4, C5a, and Bb fragments).
* Samples for the measurement of interleukin-6 in addition to CRP (as detailed below) will be sent for analysis to an appropriate laboratory and results available within a suitable timeframe in order to determine appropriate management, treatment and care for the participant as critical markers for early indicators of CRS.
* The CRP, an acute-phase reactant produced by the liver, is recognized as a marker of CRS severity even if non-specific. CRP samples will be assayed by a lab as soon as possible.
* Analysis of complement samples will be undertaken by an appropriate laboratory. The outcome of complement analysis will not be utilised for the purposes of determining appropriate management, treatment and care for the participant; however, results will be used for further understanding of the mechanisms by which CRS may be triggered in the context of this IMP. Therefore, it is not required that results of complement analysis are available to determine participant treatment in the event of potential CRS.
* Blood samples for monitoring the impact on major organs should be considered: serum creatinine, liver transaminases, total cholesterol, hemogram with cells count (WBC, RBC, Basophils, Eosinophils, monocytes, Neutrophils and lymphocytes) and activated partial thromboplastin time (aPTT).

In case of worst tolerance (moderate and above), subjects should be immediately transferred to the local hospital (with ICU facilities) with nasal oxygen and supporting treatment:

* Systolic blood pressure lower than 90 mmHg in supine position.
* Oxygen saturation lower of 90% with or without O2 supplementation.
* Respiratory rate > 24/minutes.
* Fever > 39.5°C.
* Neurological signs such as hallucinations or confusion, seizure dysphasia, tremor, or severe headache.
* High level of IL-6 (> 20 pg/mL) or CRP (> 40 mg/L).
* Any other organ dysfunction.

From the day of being discharged from the clinical unit (Day 4) up to the end of the study (Day 14), subjects should measure their oral temperature in the morning and the evening, and the results should be shared with the clinical site. They will keep a subject card in their wallet for the duration of the study. This card with the identification of the subject and the clinical centre (with an emergency phone number) will describe the potential risk of CRS with the tested product, the potential evoking symptoms, and the current guidance for the treatment of CRS. The subject should call the clinical site in any doubt or evoking symptoms except in case of medical emergency, as emergency care service shall be prioritized, i.e., always call 999 in a medical emergency.

In the current context of the COVID-19 pandemic, any fever should lead to a COVID-19 PCR test to eliminate a coronavirus infection and the current sanitary recommendation should be followed.

### Treatment Compliance

Each dose of IMP will be administered under medical supervision. The exact dosing time for each healthy subject will be recorded on the subject’s eCRF. The dosing sites of SC injections will be captured in the eCRF as well.

## Efficacy and Safety Variables

### Efficacy and Safety Measurements Assessed and Flow Chart

A schedule of study assessments is provided in Table 5.

Simbec-Orion personnel who have been appropriately trained will carry out study procedures.

Where more than 1 procedure is scheduled for the same time-point, the following order of priority will apply:

1. PK and PD blood sampling. Blood samples collected outside of the defined deviation windows will be recorded as protocol deviations. The allowable window is ± 1 minute for samples collected up to 30 minutes time-point (inclusive), ± 3 minutes for samples collected up to 2 hour time-point (inclusive) and ± 5 mins above 2 hour time-point.
2. Vital signs and 12-lead ECG (a window of ± 8 mins up to 1 hour timepoint (inclusive) and ± 15 mins above 1 hour in relation to the nominal time-point is allowed).
3. All pre-dose assessments may be performed within the 1 h before dosing, except pre-dose urine samples can be collected outside this window. Physical examination (both brief and full) and the injection site examination (a window of ± 15 mins in relation to the nominal time-point is allowed for all assessment time-points).

Table 5 Study Flow Chart

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Screening Visit** | **Treatment Period** | | | | | | | **Follow-up Visit** |
| **(Resident in the Clinical Unit)** | | | | | |  |
|  | **Screening** | **Admission** |  |  |  |  | **Discharge** | **Return**  **visit** | **Post Study** |
| **Study Day** | D-28 to -2 | D-1 | D1  pre-dose | D1  post-dose | D2 | D3 | D4 | D7 | D14 |
|
| **General Assessments** | | | | | | | | | |
| Informed Consent | X |  |  |  |  |  |  |  |  |
| Medical and Surgical History | X | X  update |  |  |  |  |  |  |  |
| Demographic Data | X |  |  |  |  |  |  |  |  |
| Hepatitis/HIV serology | X |  |  |  |  |  |  |  |  |
| PCR Test COVID-19 |  | X |  |  |  |  |  |  |  |
| Inclusion/Exclusion Criteria1 | X | X |  |  |  |  |  |  |  |
| Weight | X | X |  |  |  |  |  |  | X |
| Height/BMI | X |  |  |  |  |  |  |  |  |
| Vein Assessment | X | X |  |  |  |  |  |  |  |
| Urine DOA and alcohol/cotinine screen | X | X |  |  |  |  |  |  |  |
| Randomisation |  |  | X |  |  |  |  |  |  |
| IMP Administration in semi-supine position |  |  |  | X |  |  |  |  |  |
| **Safety Assessments** | | | | | | | | | |
| Physical Examination2 | X | X  (Brief) | X  (Brief) | X  (Brief) | X  (Brief) |  | X  (Brief) | X  (Brief) | X  (Brief) |
| Injection Site Examination3 |  |  | X | X | X |  | X | X | X |
| Vital Signs4 | X | X | X | X | X | X | X | X | X |
| Laboratory safety tests (biochemistry including C-reactive protein, haematology, coagulation, urinalysis and urine microscopy, if needed)5 | X | X |  |  | X |  | X | X | X |
| Thyroid Stimulating Hormone | X |  |  |  |  |  |  |  | X |
| 12-Lead ECG6 | X | X | X  (triplicates) | X |  |  | X |  |  |
| AE (including SAE) | X | X | X | X | X | X | X | X | X |
| Prior and Concomitant Medication | X | X | X | X | X | X | X | X | X |
| Record oral temperature in the subject temperature diaries7 |  |  |  |  |  |  | X | X |  |
| **Pharmacokinetic Assessments** | | | | | | | | | |
| PK plasma sampling8 |  |  | X | X |  |  |  |  |  |
| **Pharmacodynamics and Exploratory Assessments** | | | | | | | | | |
| PD blood for cytokines and chemokines9 |  |  | X | X | X | X |  | X |  |
| Blood for mRNA markers10 |  |  | X | X | X | X |  |  |  |
| Blood for PBMC isolate11 |  |  | X |  | X |  |  | X |  |
| Blood for titration of autoantibodies12 |  |  | X |  |  |  |  |  |  |

**Study Flow Chart Footnotes:**

1. Full inclusion/exclusion criteria will be checked at Screening. Eligibility will be re-confirmed on Day -1 and prior to dosing.
2. Physical examination: Full physical examination will be performed at Screening. A brief physical examination will be performed on Day -1, Day 1 (pre-dose and 2 h, 4h and 8 h post-dose), Day 2, Day 4, Day 7 and post study follow-up.
3. Injection site examinations will be performed at Day 1 (pre-dose and 2 h, 4h and 8 h post-dose), Day 2, Day 4, Day 7 and post study follow-up. Photographs of injection site reactions may be taken if grade 2 or above is reached.
4. Vital signs (supine blood pressure, heart rate and oral temperature, respiration rate) will be measured at Screening, Day -1, Day 1 (pre-dose and15 mins, 30 mins, 1 h, 2 h, 4 h, 8 h and 12 h post-dose), Day 2, Day 3, Day 4, Day 7 and Post Study follow-up.
5. Laboratory safety tests (biochemistry including C-reactive protein, haematology, coagulation, urinalysis and urine microscopy, if needed) will be performed at Screening, Day -1, Day 2, Day 4, Day 7 and post study follow-up, following an 8 hour fast.
6. 12-lead ECGs (HR, RR, PR, QRS, QT, QTcF) will be taken at Screening, Day -1, Day 1 (pre-dose and15 mins, 30 mins, 1 h and 8 h post-dose) and Day 4. Day 1, pre-dose ECG will be taken in triplicate (for baseline), all post-dose time points will be single ECGs. An ECG can be repeated for any reason (technical in particular). The PI or sub-Investigator must review the results. At the discretion of PI or delegate, additional ECGs can be required.
7. Temperature diary will be issued at discharge on Day 4 to record oral temperatures in the morning and evening at home from evening on Day 4 to evening on Day 6 (the diary will be collected on Day 7 and the Day 7 morning temperature taken whilst in the clinical unit). A new diary will be issued on Day 7 to record oral temperatures in the morning and evening from evening on Day 7 to the evening on Day 13 (if post study follow up is conducted on Day 14). If the post study follow up is conducted on Day 13, participants will only be required to record their temperature from the evening of Day 7 until the evening of Day 12. If the post study follow up is conducted on Day 15, participants will be required to record their temperature from the evening of Day 7 to the evening of Day 13. The diary will be collected at post study and the temperature for that day will be taken whilst in the clinical unit).
8. Plasma PK samples for CpG ODN D35 will be taken (3 mL K3-EDTA tube) on Day 1 (pre-dose and 10 mins, 20 mins, 30 mins, 45 mins, 60 mins, 2 h and 4 h post-dose).
9. PD blood samples (5 mL) for serum cytokine and chemokine analysis will be collected at Day 1 (pre-dose and 8 h and 12 h post-dose), Day 2, Day 3 and Day 7. Cytokine and chemokine samples will be batched and analysed at the end of the study or at the end of each cohort. 4.5 mL blood will be collected at the same timepoint for IL-6 analysis during the study in case it is required for safety monitoring.
10. Full blood sampling for mRNA markers will be collected (2 x 2.5 mL vacutainer PAXgene® blood RNA tube) on Day 1 (pre-dose and 4 h, 8 h and 12 h post-dose), Day 2 (24 h post-dose) and Day 3 (48 h post-dose).
11. Blood sample for PBMC isolate will be collected on Day 1 (pre-dose), Day 2 and Day 7.
12. Blood sample for titration of autoantibodies (5 mL) will be collected on Day 1, pre-dose and stored for future analysis, if required.

### Demographic and Background Assessments

The following demographic and background assessments will be performed during the study at the time‑points specified in Table 5.

#### Demographics

Demographic data: age, date of birth (only year of birth is recorded in the eCRF), gender, race, ethnicity, height, weight and body mass index (BMI).

Height in metres (to the nearest cm) and weight in kg (to the nearest 0.1 kg) in indoor clothing and without shoes will be measured. BMI = body weight (kg) / [height (m)]2 will be calculated.

#### Medical and Surgical History

Relevant medical and surgical history will be recorded in the eCRF.

#### Virology Tests

Virology tests: HBsAg, HCV Ab and HIV test (antibodies to HIV-1 and HIV-2).

Virology tests will be analysed from the same serum sample for biochemistry analyses at Screening by Simbec-Orion Laboratory Services, or at an appropriate referral laboratory, using an appropriate analyser/method(s) of analyses.

#### COVID-19 PCR Testing:

A nasopharyngeal and/or oropharyngeal swab will be collected. A real-time reverse transcription polymerase chain reaction (RT-PCR) test will be performed by Simbec-Orion Laboratories Services, using the Menarini VitaPCR analyser/using an appropriate analyser/manual kit(s)/method(s) of analysis.

#### Drugs of Abuse (including Alcohol and Cotinine)

Urine DOA screen (including alcohol and cotinine):

* Alcohol
* Cotinine
* Amphetamines
* Barbiturates
* Benzodiazepines
* Cocaine
* Marijuana/Cannabis
* Methadone
* Methamphetamine (reported under Amphetamine test)
* Ecstasy (reported under Amphetamine test)
* Morphine/Opiates
* Phencyclidine
* Tricyclic Antidepressants

A **mid-stream** urine sample will be collected into a universal collection/storage container. At protocol-defined time-points, when both urinalysis and DOA/alcohol screening are required, all urine analyses will be performed from a single approximately 30 mL urine sample.

Urine samples for DOA (including alcohol and cotinine) will be analysed by Simbec-Orion Laboratory Services, or at an appropriate referral laboratory, using an appropriate analyser/manual kit(s)/method(s) of analyses.

Assessments of urine sample quality (i.e., urine sample verification/adulteration) will be performed by measuring urine creatinine for urine DOA.

#### Compliance with Inclusion/Exclusion Criteria

An Investigator will assess all participants against the study inclusion and exclusion criteria at Screening. Compliance will be re-confirmed on Day -1.

### Efficacy Assessments

Not applicable.

### Safety Assessments

Safety assessments will be performed at the time-points specified in Table 5.

#### Definitions

**Adverse Events:**

An AE is any untoward medical occurrence in a patient or clinical trial subject administered an IMP, which does not necessarily have a causal relationship with this treatment.

It can therefore be any unfavourable and unintended sign (i.e., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Definition of an AE includes worsening (in severity and frequency) of pre-existing conditions (“Medical history”) before first IMP administration and abnormalities of procedures (i.e., 12-lead ECG results) or abnormal laboratory results which are assessed as “clinically significant”.

**Clinically Significant Laboratory Procedures/Abnormalities:**

For every laboratory assessment, the Investigator will evaluate if the laboratory test result is normal or abnormal. If abnormal (after repeat test as performed), the Investigator will assess if this finding is clinically significant or not. If a laboratory parameter is abnormal and clinically significant, it should be reported as an AE.

An abnormal laboratory/procedure result must be compared with the previous value, taking into account normal values in the studied population/country.

A treatment emergent adverse event (TEAE) is a new event after the administration of the first dose of the study drug or a worsening in the condition. In the case of abnormal laboratory/procedure tests results, it is an increase in severity (clinical intensity) of the abnormality which is judged clinically significant by the Investigator.

Any abnormalities will be assessed as “clinically significant” (and therefore have to be reported as an AE) if they meet at least one of the following conditions:

* + The abnormality suggests a disease and/or organ toxicity AND this abnormality was not present at the Screening visit or is assessed as having evolved since the Screening visit,
  + The abnormality requires medical intervention or concomitant therapy,
  + Furthermore, laboratory abnormalities associated with clinical signs and symptoms will also be considered clinically significant.

When reporting an abnormal laboratory result as an AE, a clinical diagnosis should be recorded rather than the abnormal value itself, if available. However, in these cases, the AE should be recorded as the syndromic clinical diagnosis (i.e., acute pancreatitis instead of each finding separately: high levels of amylase, high levels of lipase, abdominal pain and vomiting; “hypokalaemia” rather than “decreased potassium levels”).

**Adverse Drug Reaction:**

An adverse drug reaction (ADR) is any AE where a causal relationship with the IMP is at least a reasonable possibility (possibly related, probably related or definitely related). This means that there are facts (evidence) or arguments to suggest a causal relationship between the event and the IMP (see definition of causality below).

**Serious Adverse Events:**

An SAE is defined as any untoward medical occurrence that at any dose:

* results in death;
* is life-threatening (at the time of the event): in this context refers to an AE in which the patient was at risk of death at the time of the AE; it does not refer to an AE that hypothetically might have caused death if more severe;
* requires hospitalisation or prolongation of existing hospitalisation: i.e., the AE requires at least an overnight admission or prolongs a hospitalisation beyond the expected length of stay;
* results in persistent or significant disability or incapacity: i.e., the AE resulted in a substantial disruption of the subject’s ability to conduct normal activities;
* consists of a congenital anomaly or birth defect: i.e., an AE outcome in a child or foetus of a subject exposed to the IMP before conception or during pregnancy;
* an important medical event as recognised by the PI: i.e., AE is medically significant: medical and scientific judgment should be exercised in deciding whether other situations should be considered serious events/reactions, such as important medical event that may not be immediately life-threatening or results in death or hospitalisation but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the above definition should also usually be considered as serious. In addition, any suspected transmission via a medicinal product of an infectious agent is also considered a SAE/reaction.

**Unexpected Adverse Reaction:**

An unexpected adverse reaction is an adverse reaction, whose nature, severity or outcome is not consistent with the applicable product safety reference information on the IMP i.e., the IB.

**Suspected Unexpected Serious Adverse Reactions (SUSARs):**

SUSARs are SAEs which are believed to be related to an IMP and are both unexpected (i.e., the nature or severity is not expected from the information provided in the IB) and serious. SUSARs are subject to expedited reporting to the MHRA and REC.

**Reference Documents for Expectedness Assessment:**

The reference document for expectedness assessment of SAE related to study product for the present study is the IB currently in force at the time of SAE occurrence.

#### Adverse Events

##### Recording Adverse Events

AEs will be recorded from the time of providing written informed consent until discharge from the study at the follow-up visit.

Any untoward medical event which occurs after the completion of the clinical trial and is reported by the subject to Simbec-Orion will be classified as a “post study event”. All serious post study events assessed as related to the IMP will be reported to the pharmacovigilance of the Sponsor.

During each study visit, the subject will be questioned directly regarding the occurrence of any adverse medical event according to the schedule in the source. All AEs, whether ascribed to study procedures or not, will be documented immediately in the source. This will include the date and time of onset, a description of the AE, severity, duration, actions taken, outcome and an Investigator’s current opinion on the relationship between the study drug and the event. A diagnosis and final opinion on the relationship between the study drug and the event will be provided at the end of the study by the Investigator.

Any subject who withdraws from the study due to an AE will be followed up until the outcome is determined and written reports provided by the Investigator.

##### Analysis of Events by the Investigator

Each AE is to be classified by the Investigator (in this order):

* + For severity
  + For causality
  + As serious or non-serious.

**Grading of Adverse Event Severity**

The Investigator will evaluate each event with regard to its severity. The severity of the AEs will be determined according to the Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (September 2007)[01].

In the event that a reported AE is not listed the following will be used to assess severity:

* Mild: An AE that is easily tolerated by the subject, causes minimal discomfort, and does not interfere with everyday activities
* Moderate: An AE that is sufficiently discomforting to interfere with normal everyday activities; intervention may be needed
* Severe: An AE that prevents normal everyday activities; treatment or other intervention usually needed
* Life-Threatening: The subject is at significant risk of life; it does not refer to an event which hypothetically might have caused death if it were more severe (life threatening consequences, urgent intervention required).
* Death: Death related to an event.

When the severity of an AE changes over time, only one AE and the maximum severity will be recorded in the eCRF for each separate event. If the AE resolves but then recurs, each will be recorded as a separate AE, with the appropriate start and stop times.

To ensure no confusion or misunderstanding of the difference between the terms “serious” and “severe,” which are not synonymous, the following note of clarification is provided:

The term “severe” is often used to describe the severity of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as “serious,” which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient’s life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations**.**

**Assessment of Causality**

For both serious and non-serious AEs, the Investigator is required to assess the possible relationship between the AE and the study drug (i.e., to determine whether there exists a reasonable possibility that the study drug caused or contributed to the AE).

This means that there are facts (evidence) or arguments to suggest a causal relationship.

To help Investigators with the decision binary tree yes/no (i.e., Related/Not related) in the evaluation of causality, the Council for International Organizations of Medical Sciences (CIOMS VI) group recommends that Investigators be asked to consider the following before reaching a decision:

* Medical history (including presence of risk factors)
* Lack of efficacy/worsening of existing condition
* Study medications
* Other medications (concomitant or previous)
* Withdrawal of study medication, especially following trial discontinuation/end of study medication
* Erroneous treatment with study medication (or concomitant)
* Protocol related procedure

The terms for reporting are:

* Definitely related. The AE and administration of study agent are related in time, and a direct association can be demonstrated.
* Probably related. The AE and administration of study agent are reasonably related in time, and the AE is more likely explained by study agent than other causes.
* Possibly related. The AE and administration of study agent are reasonably related in time, and the AE can be explained equally well by causes other than study agent.
* Probably not related. A potential relationship between study agent and the AE could exist (i.e., the possibility cannot be excluded), but the AE is most likely explained by causes other than the study agent.
* Not related. The AE is clearly explained by another cause not related to the study agent.

Note: When compared to Binary “regulatory” classification, “not related” corresponds to “not related, probably not related” and “related” corresponds to “possible, probable and definitely related”.

**Adverse Event Seriousness Assessment**

The Investigator will evaluate the seriousness of any event as per the definition in Section 10.7.4.1.

##### Analysis of Events by the Sponsor

The Sponsor will also evaluate the seriousness of all events which are reported by the Investigator, and the causality of the study drug and any other treatments for each AE.

AEs for which the Investigator consider that a causal link with the study product is a reasonable possibility will be considered to be suspected adverse effects. Should the evaluations of the Sponsor and the Investigator differ with regard to causality and the event being serious, then both will be reported in the declaration of suspected adverse reactions.

The Sponsor is responsible for determining the expectedness of the SAE, using the IMP reference safety information, which is the IB currently in force at the time of SAE reporting. Each SAE has to be classified by the Sponsor as expected or unexpected for the IMP.

##### Reporting Serious Adverse Events

The Investigator is required to notify the study Sponsor and DNDi PV within 24 h of becoming aware of the occurrence of an SAE. A copy of the written report of the SAE should promptly be sent to the study Sponsor, in accordance with International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines[12].

The Investigator will notify the Sponsor without delay on the day of discovery of any SAEs.

The Investigator must:

* note in the participant's medical file the date on which he/she become aware of the SAE (at a follow-up visit or a telephone contact with the participant or a third person, etc.);
* complete the SAE form and send it by email **to pharmacovigilance@dndi.org (copy SAE\_DNDICPG@dndi.org),** immediately after of being informed of this event, without waiting for the results of the clinical outcome or additional investigations, and in any case, within 24 h of knowledge by the Investigator; this form includes a description of the event, onset date and seriousness criteria, duration, severity, causal relationship to study drug, outcome, measures taken and all other relevant clinical and laboratory data;
* make a telephone call after the initial email notification (within 24 hours of awareness of SAE) for all treatment emergent SAEs to the Sponsor Medical Responsible and/or Senior Clinical Manager.
* provide the persons designated above, as they become available, additional information (follow-up SAE form) with all relevant information that could contribute to the clarification of the SAE and to the assessment of potential risk for the study subjects and with anonymised copies of the documents which provide additional useful information, such as hospital admission reports, reports of further consultations, laboratory test reports, reports of other examinations aiding diagnosis (where possible, the results from pre-treatment assessments should be appended for comparison with the results obtained under treatment), or the autopsy report, if autopsy is performed; any follow-up reports should be submitted as soon as possible, and if possible within 2 working days of knowledge, inform the persons designated above of the outcome, if not previously reported, and other relevant follow-up information of the SAE as soon as possible;

The Investigator must also report all SAEs in the source by filling in the AE form. Where the same data are collected in the source and in the SAE form, the two forms must be completed in a consistent manner, and the same medical terminology should be used.

If the SAE is the reason of subject drop-out from the study, the Investigator will detail the reason for such a statement in the comment section of the form and the Sponsor Medical Responsible and Sponsor Clinical Project Manager will be informed immediately (within 24 h of the Investigator becoming aware of the event) by telephone and email.

The minimum criteria to be reported are as follows:

* a suspected IMP,
* an identifiable subject (at least study subject identification code number but no subject initials),
* an AE assessed as serious,
* an identifiable reporting source.

The outcome of the SAE shall be classified as following:

* recovered/resolved,
* recovering/resolving,
* recovered/resolved with sequelae,
* not recovered/not resolved,
* fatal,
* unknown.

Details should be given for the latter four categories.

* Start date of SAE or date when the AE becomes serious. SAE end date is the date of AE recovery.

##### Reporting of SUSARs

The Sponsor is responsible for all declarations to Health Authorities (MHRA) as described in this protocol and in the safety management plan (SMP). Simbec-Orion is responsible to inform the Sponsor of any update/modification of the local requirements.

It is the responsibility of the Sponsor to determine whether a reported SAE fits the classification of a SUSAR and to notify the Investigator of their decision as soon as possible.

The Sponsor (the PV service provider [United BioSource Corporation]) is responsible for reporting SUSARs to the REC and MHRA.

##### Expedited Reporting of Events

It is the responsibility of the Sponsor to determine whether an event requires expedited reporting and to notify the Investigator of their decision as soon as possible.

Where expedited reporting is required, the following procedures should be followed:

**Fatal or life-threatening SUSARs**

It is the responsibility of the Sponsor to report fatal or life-threatening SUSARs to the MHRA as soon as possible, but no later than 7 calendar days after the Sponsor first became aware of the reaction. Any additional relevant information should be sent within 8 days of the report. This responsibility may be delegated to the DNDi PV provider.

The Sponsor (represented by the pharmacovigilance service provider [United BioSource Corporation] by delegation) is required to notify the REC of any fatal or life-threatening SUSAR as soon as possible, but no later than 7 calendar days after the Sponsor first became aware of the reaction. Any additional relevant information should be sent within 8 days of the report.

**Other SUSARs (not fatal or life-threatening)**

It is the responsibility of the Sponsor to report other SUSARs to the MHRA and EMA as soon as possible, but no later than 15 calendar days after they first became aware of the reaction. This responsibility may be delegated to the PV provider.

The Sponsor (represented by the PV service provider [United BioSource Corporation] by delegation) is required to notify the REC of other SUSARs as soon as possible, but no later than 15 calendar days after they first became aware of the reaction.

##### Reporting of Urgent Safety Measures

Simbec-Orion is required to inform the appropriate competent authorities and the REC within 3 calendar days of the urgent safety measure via substantial amendment after consultation with DNDi.

##### Serious Breaches

It is the responsibility of the Sponsor to notify the licensing authority of any serious breach, which is likely to affect, to a significant degree, the safety or mental integrity of the subjects of the study or the scientific value of the study. This has been delegated to Simbec-Orion.

All serious breaches will be notified to the MHRA within 7 days. The reporting will be performed by the party who suspects the serious breach.

The personal data of volunteers will be pseudonymised in that they will only include health, date of birth (year of birth only) and demographics (gender and ethnicity) and cannot be linked back to the individual by the recipient. The Sponsor shall be the data controller in respect of the personal data of the study subjects collected in connection with the study and shall act in accordance with the relevant data protection laws in relation to the collection and processing of those personal data. The study subjects’ pseudonymised personal data shall be collected and processed for the purposes of the study and may also be added to research databases and used in the future by the Sponsor and its affiliates for certain additional clinical research, for product regulation and safety reporting purposes and for ensuring compliance with legal requirements. The study subjects’ pseudonymised personal data may be processed for such purposes by other parties including: the Sponsor’s affiliates and licensing partners, its business or academic partners, its donors, regulatory agencies and other health authorities, and ECs. The study subjects’ authorisation for such use and disclosure shall be obtained by the study subjects signing the ICF for the study.

Additionally, Simbec-Orion personnel are contractually bound by a duty of confidentiality and receive training in this matter.

##### Data Security Breach

Simbec-Orion has a comprehensive process in place for identifying, assessing, resolving and reporting any potential data security breach. All staff are trained in the identification of potential data security breaches. Potential breaches are managed by appropriately trained quality assurance (QA) personnel in accordance with Simbec-Orion SOPs. After robust assessment of data breaches, those deemed serious will be reported to the Sponsor and Information Commissioner’s Office, as applicable.

##### Monitoring of Subjects with Adverse Events

In the event of any abnormalities considered to be clinically significant by the investigating physician or ongoing AEs, subjects will be followed up with appropriate medical management until:

* It has resolved/returned to normal or baseline.
* The event has stabilised at a level acceptable to the Investigator and is not considered to be clinically significant.

#### Pregnancy

The following procedures should be followed if the partner of a subject becomes pregnant.

* Subjects will be instructed that if their partner becomes pregnant during the study, this should be reported to the Investigator who will evaluate the date of pregnancy start (1st day of last menstruation period) and if there was exposure during pregnancy based on product’s half-life. The Investigator should also be notified of pregnancy occurring during the study but confirmed after completion of the study.
* In the event that a subject’s partner is subsequently found to be pregnant after the subject has been dosed, the Investigator must submit the event in writing, on a “Pregnancy Surveillance Form”, to the Sponsor in an expedited manner, i.e., within 24 h, with the same procedure and timelines as for SAEs (see Section 10.7.4.2.4). This must be done irrespective of whether an AE has occurred. The information submitted should include the anticipated date of delivery.
* Consent will be sought from subject’s partner and, if granted, any pregnancy will be followed and the status of mother and/or child will be reported to the Sponsor after delivery or pregnancy termination (i.e., induced/spontaneous abortion).
* The Investigator will provide pregnancy outcome information on a “Pregnancy Surveillance Form”.
* In the case of a live birth, a medically qualified person should assess the infant at the time of birth and submit a “Child Surveillance Form” (provided by the Sponsor). An SAE should be declared in the case of unfavourable pregnancy outcome (abortion, still birth) or congenital abnormality (in addition to the “Child Surveillance Form”).
* In case of in utero exposure, the parents will be proposed a follow-up of the new-born up to the age of 2 years old.

Consent to report information regarding these pregnancy outcomes should be obtained from the mother.

#### Laboratory Safety Assessments

Laboratory safety screen samples will be analysed by Simbec-Orion Laboratory Services. Printed laboratory test result reports will include normal reference ranges. A decision regarding whether the laboratory test result outside the normal reference range is of clinical significance or not shall be made by an Investigator/designee and the report will be annotated accordingly. Clinically significant laboratory test result abnormalities will be recorded on the AE page. The normal reference ranges for laboratory test parameters will be detailed in the SLPDoc0024 Normal Reference Ranges and Alert Reference Values document.

##### Biochemistry Tests

* Alanine Aminotransferase (ALT)
* Albumin
* Alkaline Phosphatase
* Aspartate Aminotransferase (AST)
* Bicarbonate
* Bilirubin (Total)
* Bilirubin (Direct) (only if Total is elevated)
* Calcium
* Cholesterol (Total)
* Chloride
* C-reactive protein (CRP)
* Creatine Kinase (CK) or creatine phosphokinase (CPK)
* Creatinine
* Gamma Glutamyl Transferase (GGT)
* Glucose
* Glucose (Fasting)
* Potassium
* Phosphate (Inorganic)
* Protein (Total)
* Sodium
* Triglycerides
* Urea
* Uric Acid

Blood samples for biochemistry analyses for each time-point will be collected into an appropriately sized serum collection tube with or without a separator, and analysed by Simbec-Orion Laboratory Services, or at an appropriate referral laboratory, using an appropriate analyser/method(s) of analyses. Assessments of blood sample quality (i.e., for sample verification) will be performed by measuring 3 indices [namely, Lipaemic (for Lipaemia), Haemolytic (for Haemolysis) and Icteric (for Icterus)] in serum.

##### Thyroid Stimulating Hormone (TSH)

TSH testing will be included in biochemistry panel at Screening and Post study.

##### Haematology Tests

* Erythrocyte sedimentation rate (ESR)
* Haemoglobin
* Haematocrit (Packed Cell Volume- PCV)
* Mean Corpuscular Volume (MCV)
* Mean Corpuscular Haemoglobin (MCH)
* Mean Corpuscular Haemoglobin Concentration (MCHC)
* Platelet Count
* Red Blood Cell Count (RBC)
* White Blood Cell Count (WBC)
* WBC Differential Count (Neutrophils, Lymphocytes, Monocytes, Eosinophils, and Basophils) (Reported in absolute and percentage values).

Blood samples for haematology analyses for each time-point will be collected into an appropriately sized blood collection tube containing ethylenediaminetetraacetic acid (EDTA) and analysed by Simbec-Orion Laboratory Services, or at an appropriate referral laboratory, using an appropriate analyser/method(s) of analyses.

##### Coagulation Tests

* Activated partial thromboplastin time (aPTT)
* Prothrombin time (PT)
* international normalised ratio (INR)

Blood samples for coagulation analyses for each time-point will be collected into an appropriately sized plasma collection tube containing buffered trisodium citrate solution (0.105 M or 0.109 M, equivalent to 3.2% trisodium citrate) and analysed by Simbec-Orion Laboratory Services, or at an appropriate referral laboratory, using an appropriate analyser/method(s) of analyses.

##### Urinalysis Tests

* Bilirubin
* Blood
* Glucose
* Ketones
* Leukocytes
* Nitrites
* pH
* Protein
* Specific gravity
* Urobilinogen
* Urine Microscopy (if needed or at the discretion of Investigator based on urinalysis results).

A mid-stream urine sample for each time-point will be collected into a 30 mL collection/storage container. Urinalysis will be performed by Simbec-Orion Laboratory Services, or at an appropriate referral laboratory, using an appropriate analyser/ manual kit(s)/method(s) of analyses.

In the event that the urinalysis ‘dipstick’ test result is positive for nitrite and/or 2+ or more reported for protein, blood, and/or leucocytes, then urine microscopy will be performed by reflex. The following test parameters will be reported: bacteria, casts (non-pathogenic), casts (pathogenic), crystals, epithelial cells, red blood cells and white blood cells. The urine microscopy will be performed by Simbec-Orion Laboratory Services, or at an appropriate referral laboratory, using an appropriate analyser/method(s) of analyses.

#### Vital signs

Supine systolic/diastolic blood pressure, pulse rate, oral temperature and respiration rate.

Measurements will be recorded in the supine position after 10 min supine. Blood pressure, pulse and oral temperature will be measured by the DINAMAP\* Compact Vital Signs Monitor (Model TS) or equivalent. Normal ranges for vital signs are presented in Appendix 1.

#### Physical Examination

A physical examination will be performed by an Investigator. The full examination will include ear/nose/throat, ophthalmological, dermatological, cardiovascular, respiratory, gastrointestinal, CNS, lymph nodes and musculoskeletal. An Investigator can examine other body systems if required, at their discretion.

A brief physical examination will include respiratory, cardiovascular, GI and other examinations, if required.

#### 12-lead ECG

HR, PR interval, RR interval, QRS width, QT interval and QT interval corrected using Fredericia’s formula (QTcF).

12-lead ECG recordings will be made using a Mortara ELI280 or equivalent. Each ECG trace should be labelled with the study number, subject number, gender, age and race. An Investigator will provide an interpretation of each tracing within 2 hours. Clinically significant abnormalities will be recorded on the AE page. Normal ranges for 12-lead ECG parameters are presented in Appendix 1.

#### Concomitant Medication

All prior and concomitant medications taken during the study will be recorded in the participant’s eCRF (see Section 10.6.10).

#### Injection Site Examination

Injection site reactions (redness, swelling, warmth, tenderness, pain) will be assessed during the study, according the assessment scales in Appendix 3. Photographs of injection site reactions will be taken if grade 2 or above is reached.

### Appropriateness of Measurements

All measurements performed in the study are standard measurements.

The total volume of blood to be collected from each participant during the study (less than 340 mL) is considered acceptable (Table 6).

Table 6 Summary of Blood Volume

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Procedure** | **Visit** | **Number of Samples** | **Blood Volume per Sample (mL)** | **No. Treatment Periods** | **Blood Volume (mL)** |
| **Biochemistry** | Screening1 | 1 | 4.5 | N/A | 4.5 |
| Day -1/ Day 2/Day 4/Day 7 | 4 | 4.5 | N/A | 18 |
| Post study | 1 | 4.5 | N/A | 4.5 |
| **Haematology** | Screening | 1 | 3.0 | N/A | 3.0 |
| Day -1/ Day 2/Day 4/Day 7 | 4 | 3.0 | N/A | 12 |
| Post study | 1 | 3.0 | N/A | 3.0 |
| **ESR** | Screening/Day -1/Day 2/Day 4/Day 7/Post study | 6 | 5 | N/A | 30 |
| **Coagulation** | Screening | 1 | 2.7 | N/A | 2.7 |
| Day -1/ Day 2/Day 4/Day 7 | 4 | 2.7 | N/A | 10.8 |
| Post study | 1 | 2.7 | N/A | 2.7 |
| **PD Blood samples for cytokine and chemokine analysis** | Treatment Period | 6 | 5 | N/A | 30 |
| **Blood for IL-6 analysis in case required for safety monitoring** | Treatment Period | 6 | 4.5 | N/A | 27 |
| **PK Drug Conc. Measurement** | Treatment Period | 8 | 3 | N/A | 24 |
| **PD Blood for mRNA markers** | Treatment Period | 6 | 2 x 2.5 | N/A | 30 |
| **Blood for PBMC isolate** | Treatment Period | 3 | 40 | N/A | 120 |
| **Blood for titration of autoantibodies** | Treatment Period | 1 | 5 | N/A | 5 |
| Total Blood Volume2 | | | | | 327.2 Approximately 340 mL (+ 5%) |

1 From the biochemistry blood sample collected at the Screening visit, virology screen will be analysed from the same serum sample.

2 Please note: This total blood volume does not include any additional blood sample collection(s) for retest, unscheduled testing or additional tests required at the discretion of the Investigator/designee. The exact volumes of each sample may change but the total volume of blood drawn for any participant will not exceed 500 mL.

PD = pharmacodynamic PK = pharmacokinetic PBMC = peripheral blood mononuclear cells mRNA = messenger Ribonucleic Acid

### Primary Efficacy Variable(s)

N/A.

### Pharmacokinetic Drug Concentration Measurements

PK plasma samples for CpG ODN D35 concentration measurements will be analysed at Oncodesign, using a validated liquid chromatography tandem mass spectrometry (LC MS/MS) method, according to applicable local SOPs.

Blood sample (3 mL) for the determination of plasma CpG ODN D35 levels will be collected into K3-EDTA tube(s) per time-point as specified in Table 5.

Full details of sample handling and processing can be found in the sample handling manual (SHM).

A repeat of an already collected PK sample is not permitted during the study.

### Pharmacodynamic Assessments

Blood sample (5 mL) for the determination of serum cytokine and chemokine levels (CXCL10, IFN-γ, IL-6, optional: IFN-α, MIP1α, IL-10, TNFα and/or other biomarkers) will be collected per time-point as specified in Table 5.

4.5 mL blood will be collected at the same timepoint for IL-6 analysis during the study in case it is required for safety monitoring.

Full details of sample handling and processing can be found in the SHM.

Cytokine and chemokine levels will be analysed and reported within the study final report.

### Blood for mRNA markers

2 x 2.5 mL whole blood will be collected from the forearm vein using the vacutainer PAXgene® blood RNA tubes at the time-points in Table 5.

* Tubes will be inverted 8-10 times after collection. After a minimum of 2 hours at room temperature, and without further processing, the full blood samples will be stored at -80°C prior to shipment for analysis.

Full details of sample handling and processing can be found in the SHM.

mRNA markers will be analysed and reported separately.

### Blood for PBMC isolate

Blood samples for PBMC isolate will be collected during the study at the timepoints specified in Table 5.

Blood samples (40 mL [4x10 mL samples]) for PBMC activity assessment will be taken into sodium heparin plasma tubes at each time point, processed as described in the Laboratory Service Plan (LSP). Purified, frozen PBMCs will be shipped to a laboratory contracted by DNDi for exploratory analysis according to applicable local SOPs.

PBMC samples will be analysed and reported separately.

### Blood for Titration of Autoantibodies

Blood samples for titration of autoantibodies (5 mL) will be collected during the study, at the timepoints specified in Table 5, and will be stored for future analysis, if required.

Full details of sample handling and processing can be found in the SHM.

## Data Quality Assurance

At the time the study is initiated, a representative of the Sponsor will thoroughly review the final protocol and eCRFs with the PI and site staff. During the course of the study, the Monitor will visit the Clinical Unit regularly to check the completeness of the participants’ records (including the volunteer (participant) master files, laboratory and 12-lead ECG print-outs), the accuracy of entries into the eCRFs, the adherence to the final protocol and to ICH GCP E6 (R2) guidelines[03], the progress of enrolment and also to ensure the storage, handling and accountability of the IMP. The PI and key study personnel will be available to assist the Monitor during these visits.

The PI will give the Monitor, Auditor(s), the REC, and the MHRA direct access to relevant clinical records to confirm their consistency with the eCRF entries. No information in these records about the identity of the participants will leave Simbec-Orion. The Sponsor will maintain the confidentiality of all participant records, in line with ICH GCP E6 (R2) guidelines[03].

Study data will be fully documented in the eCRFs and study logbooks. Dated signatures will be given to account for all interventions in the study by research staff.

Source data is all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical study necessary for the reconstruction and evaluation of the study. Source data are contained in source documents (original records or certified copies).

For the purposes of this study the source data will be recorded as detailed in Table 7.

Table 7 Summary of Source Documentation Location

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Data** | **eCRF1** | **Paper Source Document** | | |
|  | **Volunteer Master File** | **Paper Source File** | **Other** **Study Documentation** |
| Evidence of healthy participant status/primary disease condition for entry into clinical study |  | X |  |  |
| Demographic data | X |  |  |  |
| Medical history and age | X | X |  |  |
| Inclusion and exclusion criteria |  |  | X |  |
| Informed consents2 |  |  | X |  |
| Participant involvement in the clinical study | X |  |  |  |
| Screening number |  |  |  | X (screening log) |
| Participant number |  |  |  | X (enrolment log) |
| AEs | X |  |  |  |
| SAEs |  |  |  | X (SAE form) |
| Pregnancies |  |  |  | X (Pregnancy notification and outcome form) |
| Previous and on-going therapy |  | X |  |  |
| Concomitant medication | X |  |  |  |
| Results of study examinations (e.g., 12-lead ECGs and laboratory safety tests)3 |  |  | X |  |
| Vital Signs | X |  |  |  |
| Physical Examination4 | X |  |  |  |
| Study visit dates | X |  |  |  |
| COVID-19 PCR test collection time |  |  |  | X (Test Request Forms) |
| Administration of IMP | X |  |  |  |
| Blood PK sample collection times | X |  |  |  |
| Blood PD/exploratory sample collection times | X |  |  |  |
| Blood safety sample collection times |  |  |  | X (Test Request Forms) |
| Urine safety sample collection times |  |  |  | X (Test Request Forms) |
| Injection site assessment | X |  |  |  |
| Photographs of injection site reactions |  |  | X |  |
| Oral Temperature (Day 4 to Day 6 and Day 7 to post study) |  |  |  | X (subject temperature diaries) |

1. In the event staff are unable to enter data directly into the eCRF (e.g., technical/internet issues), data will be entered directly into a back-up paper source workbook at the time of assessment, then transcribed and subsequently QC’d.
2. The original informed consent forms will be maintained in the study officer file during the clinical phase and will then be transferred to the Project Manager for archiving with the Investigator Site File at the end of the study.
3. The 12-lead ECG trace and laboratory safety test print-out including medical review will be stored in the paper source document file.
4. In the eCRF, the date and time of each physical examination will be recorded. Any abnormal findings will be captured on the Medical History form at Screening and as an Adverse Event during clinical conduct.

AE =adverse event, eCRF = electronic case report form, ECG = electrocardiogram, IMP = investigator medicinal product, PK = pharmacokinetic, PD = pharmacodynamic, SAE = serious adverse event

The above table indicates where source data will be recorded but for completeness, the following information will also be recorded in the volunteer master file:

* Clinical study code.
* Study visit dates (pre-dose; post-dose).
* IMP administration (date of last dose).
* Results of any key safety and efficacy measures from the clinical study that, in the opinion of an Investigator, should be noted.
* Any concomitant medications used to treat the participant during the study.

The data collected in the eCRFs during the study will be subject to quality control checking by clinical staff prior to sign off.

Designated investigator site staff will enter the data required by the protocol into the eCRF using fully validated software that conforms to 21 CFR Part 11 requirements. Staff will not be given access to the eCRF until they have been trained. Automatic validation programs check for data discrepancies and, by generating appropriate error messages, allow the data to be confirmed or corrected before transfer of the data to the Biometrics group. The Investigator must certify that the data entered into the eCRF are complete and accurate.

The study will be subject to an independent audit by the Simbec-Orion Quality Assurance Unit as outlined in Simbec-Orion SOP GRP-QA 002.

Independent clinical QA audits may be performed at any time during or following completion of the study by the Sponsor, or its authorised agents, and Regulatory Authorities and/or the REC.

## Statistical Methods and Determination of Sample Size

### Statistical and Analytical Plan

A SAP will be written by Simbec-Orion and agreed by DNDi prior to the locking of the database and subsequent reporting of the study data.

### Study Endpoints

#### Primary Endpoints

Number and severity of treatment related AE, safety laboratory parameters, vital signs, physical examination including injection site examination, ECG parameters and injection site reactions.

* Systemic AEs (including, but not limited to: fever, chills, headache, muscle aches, fatigue, nausea, vomiting, diarrhea, and joint pain).
* Safety laboratory parameters (biochemical and haematological parameters, CRP, erythrocyte sedimentation rate, urinalysis and coagulation).
* Vital signs (supine heart rate, systolic and diastolic blood pressure), oral temperature, respiration rate).
* Physical examination and injection site examination.
* 12-lead ECG (HR, RR, PR, QRS, QT, QTcF).
* Injection site reactions (redness, swelling, warmth, tenderness, pain with arm movement).

#### Secondary Endpoints

PK blood samples will be taken on Day 1 pre-dose and 10, 20, 30, 45, 60 minutes, 2 h and 4 h post-dosing. Plasma CpG ODN D35 concentrations will be measured using a LC-MS/MS assay method**.**

The following PK parameters will be derived from plasma CpG ODN D35 concentrations (additional parameters may be derived, if applicable)**:**

* Cmax (ng/mL): observed maximum plasma concentration.
* Tmax (h): first time to reach Cmax.
* λz (1/h): apparent first order terminal elimination rate constant.
* t1/2 (h): plasma elimination half-life.
* AUClast (ng.h/mL): AUC from 0 to the time of the last quantifiable concentration.
* AUCall (ng.h/mL): AUC from 0 to the time of the last observation, regardless of whether the last concentration is measurable or not.
* AUC0-inf (ng.h/mL): AUC extrapolated to infinity.
* AUC% extrapolated (%): residual area.

#### PD and Exploratory Endpoints

* PD endpoints:
  + Explore changes from baseline levels of serum cytokine and chemokine parameters (CXCL10, IFN-γ, IL-6) in serum at pre-dose and 8 h, 12 h, 24 h, 48 h post-dose and on Day 7.
  + Other relevant cytokines/chemokines may be assessed if a multiplex assay can be implemented (IFN-α, MIP1α, IL-10, TNFα and/or other parameters) at pre-dose and 8 h, 12h, 24 h, 48 h post-dose and on Day 7.
* PBMC samples:
  + Blood samples at pre-dose, Day 2 and Day 7 will be used to isolate PBMCs that will further be sent to the University of Tokyo for exploratory PD investigations.
* mRNA markers:
  + Blood sample for mRNA markers will be collected on Day 1 at pre-dose, and 4 h, 8 h, 12 h, 24 h and 48 h post-dose and will be stored for subsequent exploratory analysis on cytokine and chemokine gene expression**.**

### Analysis Sets

**Safety Set:** All randomised participants who receive IMP will be included in the safety analysis.

**PK Set:** Participants will be assigned to the PK Set on a per treatment basis. Randomised participants will be assigned to the PK Set for a particular treatment where they have received the specific treatment and comply with the following criteria:

* Do not have a pre-dose concentration that is greater than 5% of the corresponding Cmax;
* Have at least one post-dose PK sample with concentration above the lower limit of quantitation (LLOQ);
* Do not violate the protocol in such a way that may invalidate or bias the results (major protocol violators).

### Description of Statistical Methods

All statistical analysis will be performed using SAS® (the most up to date version will be used and this will be documented in the SAP).

#### Demographic and Background Data

All demographic and background data will be listed, in addition:

**Disposition:** Participant disposition will be listed with any withdrawals flagged. Frequencies (number and %) of the total number of participants dosed, completed and prematurely discontinued (including reason for discontinuation) from the study will be summarised. Additionally, the frequency of participants within each analysis set will be summarised.

**Demographics:** Demographic data will be listed. Descriptive statistics (number of participants in the analysis set (N), number of participants with non‑missing observations (n), mean, standard deviation (SD), minimum, median and maximum) will be tabulated for the continuous variables age, height, weight and BMI and frequencies (number and %) for the categorical variable race by treatment group.

#### Efficacy Data

N/A

#### Safety Data

All safety data will be listed, in addition:

**AEs:** All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary. The most up to date version that is available at the time of database build will be used and will be listed in the data management plan (DMP). The MedDRA dictionary will not be updated during the course of the study.

All AEs, including those which occurred prior to the first dose of IMP, will be listed. TEAEs i.e., existing conditions that worsen or events that occur during the course of the study after administration of IMP, will be included within the summary tables and summarised by treatment group. Treatment related TEAEs and systemic TEAEs will be summarised by treatment group.

An overall summary of AEs will be produced including the number of TEAEs; the number and % of participants reporting at least 1 TEAE, serious TEAE, TEAE leading to withdrawal from the study; the number and % of participants reporting TEAEs by severity and relationship to IMP.

The number of TEAEs and the number and % of participants reporting at least 1 TEAE will be tabulated by system organ class (SOC) and preferred term. A participant reporting multiple episodes of a particular AE within a treatment period will only contribute 1 count towards the corresponding SOC and preferred term.

The number of TEAEs and the number and % of participants reporting at least 1 TEAE will be tabulated by preferred term and sorted by descending frequency on the total number of participants with that AE. A participant reporting multiple episodes of a particular AE within a treatment period will only contribute 1 count towards the corresponding preferred term.

In addition, the number and % of participants reporting TEAEs will be tabulated by maximum severity and strongest relationship to IMP. For the summary of TEAEs by severity, if a participant has multiple events occurring within the same SOC or preferred term the event with the highest severity will be counted. Similarly, for TEAEs by relationship to IMP, if a participant has multiple events occurring within the same SOC or preferred term, the event with the highest association to IMP will be counted.

**Laboratory Safety:** Biochemistry, haematology, coagulation, and urinalysis parameters will be listed with any out of normal range values flagged. Laboratory test results which are out of normal range will also be presented separately along with normal reference ranges. Descriptive statistics (N, n, mean, SD, minimum, median and maximum) of absolute and change from baseline (Day -1) biochemistry, haematology and coagulation values at each protocol-defined time‑point will be tabulated.

**Vital Signs:** Vital signs parameters will be listed with any out of normal range values and clinical significance flagged. Descriptive statistics (N, n, mean, SD, minimum, median and maximum) of absolute and change from baseline (Day 1 pre‑dose) values at each protocol-defined time‑point will be tabulated.

**12‑Lead ECG:** 12‑Lead ECG parameters will be listed with any out of normal range values flagged. Descriptive statistics (N, n, mean, SD, minimum, median and maximum) of absolute and change from baseline (Day 1 pre‑dose) values and clinical significance at each protocol-defined time‑point will be tabulated.

Additionally, the frequency (number and % of participants) for absolute and change from baseline QTcF values will be summarised according to the below categories:

For absolute values

* QTcF ≤ 450 msec.
* QTcF > 450 msec and QTcF ≤ 480 msec.
* QTcF > 480 msec and QTcF ≤ 500 msec.
* QTcF > 500 msec.

For change from baseline

* QTcF ≤ 30 msec.
* QTcF > 30 msec and QTcF ≤ 60 msec.
* QTcF > 60 msec.

**Physical examination**: Physical examination results will be listed.

**Injection site assessment**: Injection site reactions (redness, swelling, warmth, tenderness, pain) will be assessed during the study, according to the assessment scaled in Appendix 3, listed and summarised using frequencies (number and % of participants).

#### PK Data

***Concentration-Time Data:*** Individual plasma CpG ODN D35 concentration‑time data will be listed. Concentration‑time data will also be summarised. The descriptive statistics presented will be N, n, arithmetic mean, geometric mean, SD, coefficient of variation (CV%), minimum, median and maximum. Individual and mean concentration‑time data will also be plotted on both linear and semi‑logarithmic scales by treatment group.

For the purposes of summarising and plotting concentration‑time data, concentration value(s) below the LLOQ will be assigned a value of zero if the timepoint is prior to treatment and LLOQ/2 otherwise.

***Derived PK Data:*** The following PK parameters will be derived from plasma CpG ODN D35 concentration‑time data following SC administration of CpG ODN D35 using Phoenix WinNonlin (the most up to date version will be used and this will be documented in the SAP).

For the purposes of calculating PK parameters, concentration value(s) below the LLOQ will be assigned a value of missing.

* Cmax (ng/mL): Observed maximum plasma concentration.
* Tmax (h): first time to reach Cmax.
* λz (1/h): apparent first order terminal elimination rate constant.
* t1/2 (h): plasma elimination half-life.
* AUClast (ng.h/mL): AUC from 0 to the time of the last quantifiable concentration.
* AUCall (ng.h/mL): AUC from 0 to the time of the last observation, regardless of whether the last concentration is measurable or not.
* AUC0-inf (ng.h/mL):AUC extrapolated to infinity.
* AUC% extrapolated (%): Residual area.

**Dose Proportionality***:* Dose proportionality will be assessed by performing a regression analysis of the log-transformed Cmax, AUClast and AUC0-inf values versus the log-transformed dose using the power model with a fixed effect for log(dose). For each parameter, a point estimate and 95 % confidence interval (CI) will be calculated for the slope of the regression line. The Cmax, AUClast and AUC0-inf values will also be presented graphically.

#### PD and Exploratory Data

PD and exploratory data analysis will be detailed in SAP and exploratory data will be analysed and reported separately.

### Sample Size Calculation

The sample size chosen for this study is not based on a formal statistical estimation but is considered to be adequate to meet the objectives of the study. A sufficient number of participants will be initially screened for enrolment to ensure that the planned sample size is achieved.

# PRACTICAL CONSIDERATIONS

## Storage of Data

The ISF and associated study documentation will be archived for at least 25 years after the end of the study (last participant last visit) as per European Medicine Agency Guideline INS/GCP/856758/2018[13]. The study documentation may be transferred to an offsite storage facility during this period but will remain under the control of Simbec-Orion.

The Sponsor has delegated the set up and maintenance of the Sponsor electronic trial master file (eTMF) to Simbec-Orion. The TMF will be returned to the Sponsor at the end of the study, who will archive it for at least 25 years after the end of the study.

## Protocol Amendments

Changes in the study protocol must take the form of written protocol amendments and shall require the approval of all persons responsible for the study (see Section 1).

A protocol amendment is deemed to constitute a substantial protocol amendment if it is considered to be likely to affect to a significant degree either:

1. The safety or physical or mental integrity of the participants of the study.
2. The scientific value of the study.
3. The conduct or management of the study.
4. The quality or safety of any IMP used in the study.

Increase in dose above 180 mg would be considered substantial.

Such amendments must be submitted to the REC responsible for the study and the MHRA for approval prior to implementation.

Protocol amendments required for urgent safety reasons may be implemented immediately. However, the REC and MHRA must be notified in writing within 3 days of the measures taken and the reasons for implementation.

All other amendments shall be deemed to be non-substantial and as such, do not need the prior approval of the REC and the MHRA.

## Confidentiality

The confidentiality of the study must be maintained at all times and the PI must not reveal any information relating to the study without express permission from the study Sponsor.

## Study Report and Publication Policy

The PI will obtain the Sponsor’s written permission before any information concerning this study is submitted for publication.

## General Data Protection Regulation (GDPR)

Personal data of the participant shall be processed in a manner that ensures it has appropriate security. This includes protection against unauthorised or unlawful processing and against accidental loss, destruction or damage and by using appropriate technical or organisational measures. One such measure is by the Investigator ensuring that the participants’ personally identifiable information should be replaced through the use of pseudonymisation.

On the eCRFs or other documents submitted to DNDi/Simbec-Orion, participants will NOT be identified by their names but by the assigned participant number (panel/screening/participant number) to ensure confidentiality of the participants’ information and that data minimisation principles are maintained. If participant names are included in error on copies of documents submitted to DNDi/Simbec-Orion participants’, the names (including initials) will be erased or securely destroyed, and the assigned participant number added to the document.

# REFERENCES

1. Guidance for Industry. Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. U.S. Department of Health and Human Services Food and Drug Administration Centre for Biologics Evaluation and Research. September 2007.
2. World Health Association Declaration of Helsinki. Recommendations guiding physicians in biomedical research involving human subjects. Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the 29th WMA General Assembly, Tokyo, Japan, October 1975, 35th WMA General Assembly, Venice, Italy, October 1983, 41st WMA General Assembly, Hong Kong, September 1989, and 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996, 52nd WMA General Assembly, Edinburgh, Scotland, October 2000, 53rd WMA General Assembly, Washington 2002 (Note of Clarification on Paragraph 29 added), 55th WMA General Assembly, Tokyo 2004 (Note for Clarification on Paragraph 30 added), 59th WMA General Assembly, Seoul, October 2008 and 64th WMA General Assembly, Fortaleza, Brazil, October 2013.
3. ICH (International Council on Harmonisation) Guideline for Good Clinical Practice (GCP) E6 (R2) (CPMP/ICH/135/95) 2016.
4. Insurance and compensation in the event of injury in Phase I clinical trials 2012. Guidance developed by the ABPI (The Association of the British Pharmaceutical Industry), the BioIndustry Association and the Clinical Contract Research Association in consultation with the Department of Health and the National Research Ethics Service.
5. Haile, L. A., Puig, M., Polumuri, S. K., Ascher, J., & Verthelyi, D. (2017). In vivo effect of innate immune response modulating impurities on the skin milieu using a macaque model: impact on product immunogenicity. Journal of pharmaceutical sciences, 106(3), S. 751-760.
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7. Verthelyi, D., & Klinman, D. (2003). Immunoregulatory activity of CpG oligonucleotides in humans and nonhuman primates. Clinical Immunology, 109(1).
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10. Thacker, S. G., McWilliams, I., Bonnet, B., Halie, L., Beaucage, S., Rachuri, S., Verthelyi, D. (2020). CpG ODN D35 improves the response to abbreviated low-dose pentavalent antimonial treatment in non-human primate model of cutaneous leishmaniasis. PLOS Neglected Tropical Diseases, 14(2).
11. Investigator’ Brochures (CPG ODN D35), Version 1.0, 19 March 2021.
12. EU GMP Annex 13: Investigational Medicinal Products, https://www.gmp-compliance.org/guidemgr/files/2009\_06\_annex13.pdf
13. European Medicine Agency Guideline on the content, management and archiving of the clinical trial master file (paper and/or electronic) EMA/INS/GCP/856758/2018.

Appendix 1: NORMAL RANGES FOR VITAL SIGNS AND ECG PARAMETERS

|  |  |  |
| --- | --- | --- |
| **Vital Sign Parameters** | | |
| **Parameter** | **Normal Range** | **Units** |
| Heart Rate | 45-90 | beat(s) per minute (bpm) |
| Systolic Blood pressure | 100-140 | millimetre(s) of mercury (mmHg) |
| Diastolic Blood pressure | 50-90 | millimetre(s) of mercury (mmHg) |
| Respiratory Rate | 12-18 | breath(s) per minute |
| Oral Temperature | 35.0-37.5 | degrees Celsius (ºC) |
| Pulse Oximetry | 94-100 | percent (%) |

|  |  |  |
| --- | --- | --- |
| **ECG Parameters** | | |
| **Parameter** | **Normal Range** | **Units** |
| Heart Rate | 45-90 | beat(s) per minute (bpm) |
| PR Interval | 120-220 | millisecond(s) (ms) |
| QRS Width | 70-120 | millisecond(s) (ms) |
| QT Interval | N/A | N/A |
| QTc Interval (Fridericia’s Formula) | Male: 350-450 | millisecond(s) (ms) |

Appendix 2: DECLARATION OF HELSINKI (BRAZIL, 2013)

[https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for medical-research-involving-human-subjects/](https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for%20medical-research-involving-human-subjects/)

Appendix 3: GRADING AND MANAGEMENT OF INJECTION SITE REACTIONS GRADING OF INFUSION REACTIONS

Individual signs or symptoms (e.g., erythema, swelling, etc.) at the injection site reported by a subject following dose administration will be recorded as an injection site reaction and an AE if grade 1 or more.

Table 8: Grading of Injection Site Reactions, Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Local Reaction to**  **Injectable Product** | **Mild (Grade 1)** | **Moderate (Grade 2)** | **Severe (Grade 3)** | **Potentially Life**  **Threatening**  **(Grade 4)** |
| Pain | Does not interfere  with activity | Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity | Any use of narcotic pain reliever or prevents daily  activity | Emergency room  (ER) visit or  hospitalization |
| Tenderness | Mild discomfort to touch | Discomfort with movement | Significant  discomfort at rest | ER visit or  hospitalization |
| Erythema/Redness \* | 2.5 – 5 cm | 5.1 – 10 cm | > 10 cm | Necrosis or  exfoliative dermatitis |
| Induration/Swelling \*\* | 2.5 – 5 cm and does not interfere  with activity | 5.1 – 10 cm or  interferes with  activity | > 10 cm or  prevents daily  activity | Necrosis |

\* In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

\*\* Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.