

STUDY PROTOCOL SYNOPSIS

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:	2021-001179-18
IRAS ID:	297265
INVESTIGATIONAL MEDICINAL PRODUCT(s):	CpG ODN D35

SYNOPSIS

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 <ul style="list-style-type: none">To assess the safety and tolerability of a single subcutaneous dose of CpG ODN D35 in healthy male subjects. Secondary Objectives <ul style="list-style-type: none">To determine PK parameters of CpG ODN D35 in plasma after single subcutaneous dose in healthy male subjects. PD and Exploratory Objectives <ul style="list-style-type: none">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: <p>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.</p> <p>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.</p> <p>Each cohort will follow a sentinel dose escalation schedule.</p> <ul style="list-style-type: none">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, heart 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, heart 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, heart 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, heart rate, oral temperature and respiration rate): 15 min, 30 min, 1 h, 2 h, 4 h, 8 h 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:
 - **Cohorts 1 & 2:** 10 min, 20 min, 30 min, 45 min, 60 min, 2 h and 4 h post-dose
 - **Cohorts 3 & 4:** 5 min, 10 min, 15 min, 20 min, 25 min, 30 min, 45 min, 60 min, 2 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, heart 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, heart 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, heart 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, heart 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, heart 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 the planned study dose and had sufficient plasma samples collected to estimate, if possible C_{max} and AUC_{last} 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^[Error! Reference source not found.].

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 $100 \times 10^9/L$, lymphocyte count below $0.5 \times 10^9/L$, 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 AUC_{last} of $\geq 755 \text{ ng}\cdot\text{h/mL}$. These limits are based on the exposure observed at the 15.9 mg/kg in cynomolgus monkeys (systemic NOAEL: AUC_{last} of $755.8 \text{ ng}\cdot\text{h/mL}$ in males).
- If the dose in a single subject is anticipated to exceed a C_{max} of $\geq 1230 \text{ ng/mL}$. These limits are based on the exposure observed at the 15.9 mg/kg in cynomolgus monkeys (systemic NOAEL: C_{max} 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 $\geq 60 \text{ kg}$ to $\leq 90 \text{ kg}$, BMI 18 to 30.1 kg/m^2 . 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 $\leq 140 \text{ mmHg}$, Diastolic blood pressure $\leq 90 \text{ mmHg}$, measured after 10 min rest in supine position at Screening, admission, and pre-dose.
5. A resting heart rate (HR) between ≥ 45 and $\leq 90 \text{ bpm}$ 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 $\leq 450 \text{ msec}$.

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.

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 for Cohorts 1 & 2 and at pre-dose, 5 min, 10 min, 15 min, 20 min, 25 min, 30 min, 45 min, 60 min, 2h post-dose for Cohorts 3 & 4. 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:

- C_{max} (ng/mL): Observed maximum plasma concentration.
- T_{max} (h): first time to reach C_{max} .
- λ_z (1/h): apparent first order terminal elimination rate constant.
- $t_{1/2}$ (h): plasma elimination half-life.
- AUC_{last} (ng.h/mL): AUC from 0 to the time of the last quantifiable concentration.
- AUC_{all} (ng.h/mL): AUC from 0 to the time of the last observation, regardless of whether the last concentration is measurable or not.
- AUC_{0-inf} (ng.h/mL): AUC extrapolated to infinity.
- $AUC_{\% \text{ extrapolated}}$ (%): 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 , MIP1 α , IL-10, TNF α 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 [Error! Reference source not found.](#), 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 (C_{max}), time to C_{max} (T_{max}), terminal elimination half-life ($t_{1/2}$), apparent first order terminal elimination rate constant (λ_z), area under the concentration-time curve (AUC) from time of dosing to last measurable concentration (AUC_{last}), AUC_{all} , AUC extrapolated to infinity (AUC_{0-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 C_{max} , AUC_{last} , and AUC_{0-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 C_{max} , AUC_{last} and AUC_{0-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).

