STUDY PROTOCOL

STUDY TITLE:	A Phase I, Double-blind, Randomised, Single Centre, Parallel-group, Multiple-dose, Dose-escalation, Placebo-controlled Study of the Safety, Tolerability and Pharmacokinetics of DNDI-0690 after Oral Dosing in Healthy Subjects
STUDY NUMBER:	RD 777/34920 (DNDi- 0690- 02)
EudraCT NUMBER:	2020-003963-24
IRAS ID:	288914
INVESTIGATIONAL MEDICINAL PRODUCT(IMP):	DNDI-0690
PLANNED STUDY DOSES:	**Part A**: Cohort 1: 400 mg DNDI-0690 or placebo once a day for 10 days. Cohorts 2-4: DNDI-0690 dose to be confirmed after dose escalation review meeting. **Part B**: 3600 mg DNDI-0690 or placebo once a day for 5 days. **Part C**: DNDI-0690 dose to be confirmed.
PRINCIPAL INVESTIGATOR:	Dr Ezanul Wahab Simbec-Orion Merthyr Tydfil CF48 4DR, United Kingdom
STUDY SPONSOR:	DNDi, Chemin Louis Dunant, 15, 1202 Geneva Switzerland
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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

1 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 subjects.

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2 PROTOCOL AMENDMENT/REVISION HISTORY

Protocol Version/ Date	Type of Amendment	Amendment Rationale	Sections Affected	Summary of Amendment / Changes to the Protocol
V1.0/23 September 2020	N/A	N/A	N/A	N/A
V2.0/13 October 2020	N/A	Amendment requested in MHRA notice of grounds for non-acceptance letter dated 12 October 2020.	Section 3 (Synopsis) and Section 10.2.2 (Stopping Criteria)	The following wording: "If events are confirmed significantly more frequent in the active group, dose escalation will only proceed once an appropriate substantial amendment has been submitted and received regulatory and ethical approval from the relevant ethics committee and competent authority." have been changed to: "if any of the stopping criteria occurred in a subject receiving DNDI-0690 the trial will put on halt and further dosing can be resumed only once an appropriate substantial amendment has been submitted and received regulatory and ethical approval from the relevant ethics committee and competent authority."
V3.0/07 December 2020	Non-substantial	To document administrative changes and to correct discrepancies throughout the protocol.	Signature page, Synopsis, Section 10.7.1 (Flow Chart), Section 10.7.7.1 (PK blood sampling for DNDI-060 and metabolite), Section 10.7.10 (plasma samples for iohexol) and throughout protocol.	• Change of DNDi responsible physician (Medical Responsible) • Increased acceptable deviation window for vital signs and 12-lead ECG • Correction of Troponin baseline in Table 8 Part C (Day 1 pre-dose, not Day -1). • Post-Study vital sign added in the Table 8 Part C flowchart. • Day 4 urine PK sample removed from Table 7 Part B flowchart and synopsis. • Weight measurement removed from Day -2 in Table 8 Part C flowchart. • Part B dosing will only be OD dosing. • Footnote of Table 10: Clarification on source documentation location for subject eligibility. • Table 6 & 7, Footnote 5 has been updated. • Table 8 Footnote 10: Text clarified about Iohexol assessment at Post-study Follow-up visit. • Table 9 updated to reflect change in blood volume as tube sizes have changed. • Sample processing information updated.

				• Plasma samples for DNDI-0690 and metabolite: tube size modified (4 mL instead of 6 mL). • Blood sample for the determination of plasma iohexol levels: tube size modified (4 mL instead of 5 mL). • Added triglycerides in biochemistry panel. • Discrepancies and typos were corrected throughout the protocol.

3 SYNOPSIS

NAME OF COMPANY: Drugs for Neglected Diseases initiative (DNDi)
NAME OF INVESTIGATIONAL MEDICINAL PRODUCT: DNDI- 0690
NAME OF ACTIVE INGREDIENT: nitroimidazooxazine
TITLE OF STUDY: A Phase I, Double-blind, Randomised, Single Centre, Parallel-group, Multiple-dose, Dose-escalation, Placebo-controlled Study of the Safety, Tolerability and Pharmacokinetics of DNDI-0690 after Oral Dosing in Healthy Subjects
PRINCIPAL INVESTIGATOR: Dr Ezanul Wahab
STUDY CENTRE: Simbec-Orion Clinical Pharmacology Merthyr Tydfil, CF48 4DR, UK
CLINICAL PHASE: I

OBJECTIVES:

Part A & B

Primary Objective

- To assess the safety and tolerability of DNDI-0690 after multiple oral doses in healthy subjects in fasted conditions.

Secondary Objective

- To investigate plasma and urinary pharmacokinetics (PK) of DNDI-0690 after multiple oral doses in healthy subjects in fasted conditions.

Exploratory Objectives

- To investigate any potential changes to renal toxicity markers in urine.
- To assess the effect of DNDI-0690 on Holter electrocardiogram (ECG) parameters.
- To compare PK bioanalysis in fixed volume dry blood spots versus PK bioanalysis in plasma samples.
- To investigate the metabolite profile of DNDI-0690 after multiple dosing.
- To assess variation of mRNA expression in full blood before and after exposure to the drug (Transcriptional Profiling).

Part C

Primary Objective

- To evaluate renal function (glomerular filtration rate (GFR)) before and after administration of DNDI-0690 in healthy subjects in fasted condition at maximum well tolerated dose tested in Part A or below.

METHODOLOGY:

The study will be conducted in three parts (Part A, Part B and Part C).

Part A of the study is a single centre, double-blind, randomised, placebo-controlled, parallel-group, multiple oral dose, dose-escalation study to assess the safety, tolerability and PK of DNDI-0690 after multiple oral doses in the fasted condition in healthy male and woman of non-childbearing potential (WONCBP) subjects.

Part B of the study is a single centre, double-blind, randomised, placebo-controlled, multiple oral dose study to assess the pharmacodynamic effect of DNDI-0690 on cardiac function after multiple oral doses at the supra-therapeutic dose in the fasted condition in healthy male and WONCBP subjects.

Part C of the study is a single centre, double-blind, randomised, placebo-controlled, multiple oral dose study to evaluate the renal function (measured Glomerular Filtration Rate (mGFR)) before and after multiple oral doses in the fasted condition in healthy male and WONCBP subjects.

Part A: Multiple Ascending Dose Cohorts (Cohorts 1-4)

Part A will consist of up to 4 cohorts of 9 subjects. Subjects will be randomly assigned to receive an oral dose of active IMP (6 subjects) or matching placebo (3 subjects) for 10 days in a sequential escalating manner with a minimum of 7 days interval between two cohorts.

In Part A, each cohort will follow a sentinel dose-escalation schedule; two (2) subjects will be dosed on the first dosing day of each cohort (1 subject on active IMP and 1 subject on matching placebo). The remainder of the cohort (5 subjects on active IMP and 2 subjects on matching placebo) will be dosed a minimum of 48 hours later pending confirmation of an acceptable safety profile in the dose-leader cohort by the Principal Investigator (PI), or medically-qualified designees who are familiar with the study protocol and Investigator's Brochure (IB).

The planned starting dose for Cohort 1 is 400 mg of DNDI-0690 once a day for 10 days. Doses to be administered in Cohorts 2 to 4 will be determined based on emerging PK and safety data. The number of daily doses may be increased to 2 by implementation of a twice a day (BID) dosing regimen if the number of capsules in a single intake is raising concerns of acceptability. This decision will be made by the Safety Review Committee (SRC) prior to each cohort and will apply to all subjects within a cohort.

Dose escalation between cohorts in Part A (and between Part A and Part B) will be temporarily stopped pending evaluation of all available data if any of the following criteria are fulfilled:

- A serious adverse reaction (*i.e.* a serious adverse event (SAE) considered at least possibly related to the IMP administration) occurs in one subject.

Or

- Severe non-serious adverse reactions (*i.e.* severe non-serious AE considered as, at least possibly related to the IMP administration) occur in two subjects in the same cohort, independent of within or not within the same system organ class.

Or

- If two or more subjects in the preceding dose group experience any of the following:
    - Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >3x upper limit of normal (ULN), considered clinically significant and IMP related.
    - Serum Cystatin C (Cys C) increase > 25%, confirmed after repeat assessment 24-48h apart, and considered IMP related.
    - Ratio of microalbuminuria/creatininuria >300 mg/g and considered IMP related.
    - An increase in corrected QT interval by Fridericia's formula (QTcF) value of >60 msec from baseline (average of baseline triplicate values) or an absolute QTcF >500 msec, both confirmed by repeat measurement after 20 minutes in resting position, and considered IMP related.

Or

- If one or more subjects in the preceding dose group experience:
    - An increase in troponin I concentration which is considered clinically significant by the Investigator (with reference to the change from baseline value) and considered IMP related.

Or

- If the dose in a single subject is anticipated to exceed an AUC from time of dosing to the end of the dosing interval ($AUC_{0\text{-}tau}$) of ≥100 µg*h/mL. These limits are based on the exposure observed at the 20 mg/kg in cynomolgus monkeys (no observed adverse effect level (NOAEL): $AUC_{0\text{-}24}$ of 101 µg*h/mL in male and 111 µg*h/mL in females).

Or

- If the dose in a single subject is anticipated to exceed a maximum concentration (C_{max}) of ≥ 8.0 µg/mL. These limits are based on the exposure observed at the 20 mg/kg in cynomolgus monkeys (NOAEL: C_{max} of 8.03 µg/mL in males and 9.03 µg/mL in females).

Dose escalation between cohorts in Part A will be limited to a maximal 3-fold increase, and 2-fold increase between Part A and Part B, depending on safety and tolerability data and predicted exposure (both AUC and C_{max}).

If any of the above criteria are fulfilled, the cases will be discussed at the SRC who could proceed with partial or full unblinding as necessary to take its decision to either dose-escalate, proceed to enlargement of the cohort, or explore a lower dose.

If any of the stopping criteria occurred in a subject receiving DNDI-0690 the trial will put on halt and further dosing can be resumed only once an appropriate substantial amendment has been submitted and received regulatory and ethical approval from the relevant ethics committee and competent authority.

Each cohort will follow the same study design including Screening Period, Treatment Period and Post Study Follow-up. The study sequence for Part A is presented in Figure 1.

Screening Period (Day -28 to Day -3):

After signing the informed consent form (ICF), Screening assessments will be performed within 28 days of the planned first 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
- Vein assessment
- Urine drugs of abuse (DOA) and alcohol/cotinine screen
- Physical examination (full)
- Laboratory safety tests (biochemistry, haematology, coagulation, urinalysis, urine microscopy and follicle-stimulating hormone (FSH) for post-menopausal female subjects only)
- 12-lead ECG
- Vital signs (supine systolic and diastolic blood pressure, pulse rate, and tympanic temperature)
- Adverse event (AE)
- SAE
- Prior and concomitant medication

Treatment Period (Day -2 to Day 12):

Subjects will be admitted to the clinic in the morning of Day -2 and will remain in the unit until the 48 hours post-last dose scheduled assessments and procedures have been performed (Day 12).

Day -2:

- Polymerase chain reaction (PCR) test for COVID-19
- Eligibility check
- Urine DOA and alcohol/cotinine screen
- A brief physical examination
- Laboratory safety tests (biochemistry, haematology, coagulation, urinalysis and urine microscopy)
- Troponin I
- 12-lead ECG
- Vital signs (supine systolic and diastolic blood pressure, pulse rate, and tympanic temperature)
- AE
- SAE

- Prior and concomitant medication
- Full blood sampling for transcriptional profiling (mRNA) assessment (**Only performed on Cohort 2 and Cohort 3)**

Day -1:

- Start Holter monitoring (for extraction of ECGs) at approximately 60 minutes prior to the theoretical time of Day 1 dosing (Holter will be on for at least 25 hours and can only be removed after Day 1 dosing)
- AE
- SAE
- Prior and concomitant medication

Day 1:

Dose administrations of DNDI-0690 capsules or matching placebo will occur in the morning of Day 1 in a randomised, double-blind manner.

- Eligibility check: Pre-dose
- Subjects in once daily (OD) regimen will be dosed following an overnight fast of at least 10 hours.
- If a BID regimen is implemented, subjects in BID regimen will be dosed following an overnight fast of at least 10 hours for the morning dose, and 10 hours later for the afternoon dose. The afternoon dose shall be taken at least 5 hours post mid-day meal, and one hour before the dinner.

The specific instructions for Day 1 dosing apply for all dosing days.

The following procedures will be performed on **Day 1:**

- End Holter after morning dosing
- 12-lead ECG: Pre-morning dose (in triplicate) and 4 h post-morning dose (single)
- Vital signs (supine systolic and diastolic blood pressure, pulse rate and tympanic temperature): Pre-morning dose
- AE
- SAE
- Prior and concomitant medication
- PK plasma sample for DNDI-0690 and metabolite: Pre-morning dose and 0.5, 1, 2, 3, 3.5, 4, 6, 9, 12 h post-morning dose
- DBS (Dried Blood Spot) samples for DNDI-0690: Pre-morning dose, 0.5, 1 and 2 h post-morning dose
- PK urine samples for DNDI-0690: Pre-morning dose and [0-24] h post morning dose
- Urine samples for exploratory biomarkers: Pre-morning dose

Day 2:

- Eligibility check: Pre-dose
- Dosing
- Laboratory safety tests (biochemistry, haematology, coagulation, urinalysis, urine microscopy): Pre-morning dose
- AE
- SAE
- Prior and Concomitant Medication
- PK plasma sample for DNDI-0690 and metabolite: Pre-morning dose
- Urine samples for exploratory biomarkers: Pre-morning dose

Day 3, Day 5, Day 6, Day 8 and Day 9:

- Eligibility check: Pre-dose
- Dosing
- AE

- SAE
- Prior and Concomitant Medication
- PK plasma sample for DNDI-0690 and metabolite: Pre-morning dose

Day 4 and Day 7:

- Eligibility check: Pre-dose
- Dosing
- A brief physical examination
- Laboratory safety tests (biochemistry, haematology, coagulation, urinalysis, urine microscopy): Pre-morning dose
- 12-lead ECG: Pre-morning dose and 4 h post-morning dose
- Vital signs (supine systolic and diastolic blood pressure, pulse rate, and tympanic temperature)
- AE
- SAE
- Prior and concomitant medication
- PK plasma sample for DNDI-0690 and metabolite: Pre-morning dose
- PK urine samples for DNDI-0690:[0-24] h post-morning dose
- Urine samples for exploratory biomarkers: Pre-morning dose

Day 10:

On Day 10, only a morning dose will be administered.

- Start Holter monitoring (for extraction of ECGs) at approximately 60 minutes prior to dosing (Holter will be on for at least 25 hours and can only be removed after 24 h post-last dose PK blood samples are collected)
- Eligibility check: Pre-dose
- Dosing (morning only on Day 10, even if BID regimen implemented)
- Laboratory safety tests (biochemistry, haematology, coagulation, urinalysis, urine microscopy): Pre-dose
- Troponin I: Pre-dose
- 12-lead ECG: Pre-dose and 4 h post-dose
- Vital signs (supine systolic and diastolic blood pressure, pulse rate and tympanic temperature): Pre-dose
- AE
- SAE
- Prior and concomitant medication
- PK plasma sample for DNDI-0690 and metabolite: Pre-dose and 0.5, 1, 2, 3, 3.5, 4, 6, 9, 12 h post-dose
- DBS samples for DNDI-0690: Pre-dose and 0.5, 1, 2, 3, 3.5, 4, 6, 9, 12 h post-dose
- PK urine samples for DNDI-0690:[0-24] h post-dose
- Urine samples for exploratory biomarkers: Pre-dose
- Full blood sampling for transcriptional profiling (mRNA) assessment (**Only performed on Cohort 2 and Cohort 3**)

Day 11:

- End Holter after 24 h PK sample
- AE
- SAE
- Prior and concomitant medication
- PK plasma sample for DNDI-0690 and metabolite: 24 h post-last dose
- DBS samples for DNDI-0690: 24 h post-last dose

Day 12:

- A brief physical examination
- Weight

- Laboratory safety tests (biochemistry, haematology, coagulation, urinalysis, urine microscopy)
- 12-lead ECG
- Vital signs (supine systolic and diastolic blood pressure, pulse rate, and tympanic temperature)
- AE
- SAE
- Prior and concomitant medication
- PK plasma sample for DNDI-0690 and metabolite: 48 h post-last dose
- DBS samples for DNDI-0690: 48 h postlast dose

If all assessments are satisfactory to the PI (or deputy), subjects will be discharged from clinic after all Day 12 procedures are completed. In case of Clinically Significant laboratory results on Day 12, the volunteer will be given an appointment for an unscheduled control visit.

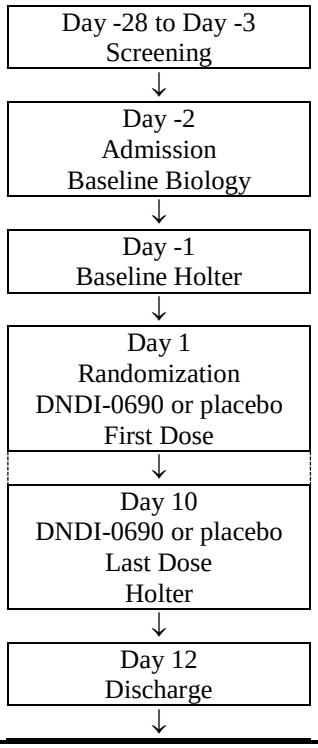
Post Study Follow-up (Day 24 to Day 28):

A post study follow-up visit will take place 14 to 18 days after last-dose (Day 24 to Day 28) to ensure the ongoing wellbeing of the subjects.

- A brief physical examination
- Laboratory safety tests (biochemistry, haematology, coagulation, urinalysis, urine microscopy)
- Vital signs (supine systolic and diastolic blood pressure, pulse rate, and tympanic temperature)
- AE
- SAE
- Prior and concomitant medication

If all follow-up assessments are satisfactory to the PI (or deputy), the subject will be discharged from the study at the discretion of the PI/deputy. 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**.**

Figure 1 Study Sequence Part A



Day 24 to 28 Follow-Up Visit

Part B: Cardiac pharmacodynamic assessment (Cohort 5 - Optional)

Part B will consist of 1 cohort of 9 subjects. Subjects will be randomly assigned to receive an oral dose of active IMP (6 subjects) or matching placebo (3 subjects) for 5 days. Part B will only be implemented if the SRC considers it safe and appropriate to proceed with a maximum considered dose of 3600 mg. This cohort is thus considered optional.

Subjects in Part B will follow a sentinel schedule; two (2) subjects will be dosed on the first dosing day (1 subject on active IMP and 1 subject on matching placebo). The remainder of the cohort (5 subjects on active IMP and 2 subjects on matching placebo) will be dosed a minimum of at least 48 hours later pending confirmation of an acceptable safety profile in the dose-leader cohort by the PI, or medically-qualified designees who are familiar with the study protocol and IB.

The study sequence for Part B is presented in Figure 2.

Screening Period (Day -28 to Day -3):

- Screening procedures are identical to Part A.

Treatment Period (Day -2 to Day 7):

Subjects will be admitted to the clinic in the morning of Day -2 and will remain in the unit until 48 h post-last dose scheduled assessments and procedures have been performed (Day 7).

Day -2: Same procedures as Day -2 of Part A.

Day -1: Same procedures as Day -1 of Part A.

Day 1 to Day 4: Same procedures as Day 1 to Day 4 procedures of Part A, except no Day 4 PK urine samples for DNDI-0690 are required

Day 5:

On Day 5, the last dose will be administered.

- Start Holter monitoring (for extraction of ECGs) at approximately 60 minutes prior to dosing (Holter will be on for at least 25 hours and can only be removed after 24 h post-last dose PK blood samples are collected)
- Eligibility check: Pre-dose
- Dosing
- Laboratory safety tests (biochemistry, haematology, coagulation, urinalysis, urine microscopy): Pre-dose
- Troponin I: Pre-dose
- 12-lead ECG: Pre-dose and 4 h post-dose
- Vital signs (supine systolic and diastolic blood pressure, pulse rate and tympanic temperature): Pre-dose
- AE
- SAE
- Prior and concomitant medication
- PK plasma sample for DNDI-0690 and metabolite: Pre-dose and 0.5, 1, 2, 3, 3.5, 4, 6, 9, 12 h post-dose
- DBS samples for DNDI-0690: Pre-dose and 0.5, 1, 2, 3, 3.5, 4, 6, 9, 12 h post-dose
- PK urine samples for DNDI-0690:[0-24] h post-dose
- Urine samples for exploratory biomarkers: Pre-dose

Day 6:

- End Holter after 24 h PK sample
- AE

- SAE
- Prior and concomitant medication
- PK plasma sample for DNDI-0690 and metabolite: 24 h post-last dose
- DBS samples for DNDI-0690: 24 h post-last dose

Day 7:

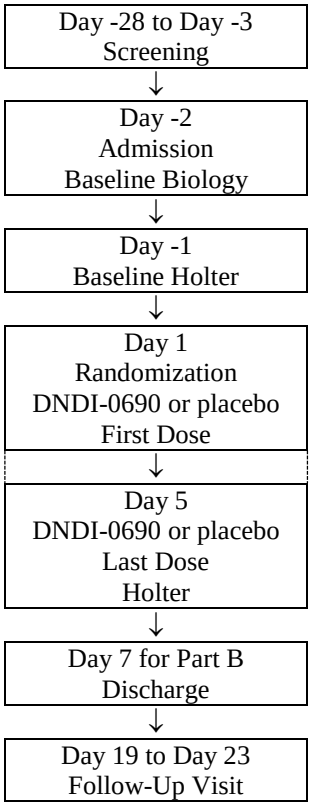
- A brief physical examination
- Weight
- Laboratory safety tests (biochemistry, haematology, coagulation, urinalysis, urine microscopy)
- 12-lead ECG
- Vital signs (supine systolic and diastolic blood pressure, pulse rate, and tympanic temperature)
- AE
- SAE
- Prior and concomitant medication
- PK plasma sample for DNDI-0690 and metabolite: 48 h post-last dose
- DBS samples for DNDI-0690: 48 h post-last dose

If all assessments are satisfactory to the PI (or deputy), subjects will be discharged from the clinic after all Day 7 procedures are completed.

Post Study Follow-up (Day 19 to Day 23):

- Post study follow-up procedures are identical to Part A.

Figure 2 Study Sequence Part B



Part C: Measured Glomerular Filtration Rate (mGFR) Cohort (Cohort 6)

Part C will consist of 1 cohort of 9 subjects.

- Subjects will be administered DNDI-0690 or placebo once daily for 10 days at a dose level that is either at or below the highest well tolerated dose of DNDI-0690 as evaluated in the Part A dose escalating cohorts. The dose will be decided by the SRC after reviewing data from Part A. As this dose will already have been explored, no sentinel group will be implemented in this cohort.
- All subjects will receive 5 mL of iohexol solution (300 mg/mL iodine) intravenously on Day -1, Day 10, and optionally Day 24-28 that will be flushed with 10 mL of normal saline solution. On Day -1, iohexol will be administered at the same time as expected dosing of the study drug or placebo on Day 10. On Day 10, iohexol will be administered immediately after dosing of study drug or placebo.

The study sequence for Part C is presented in Figure 3.

Screening Period (Day -28 to Day -3):

- Screening procedures are identical to Part A.

Treatment Period (Day -2 to Day 12):

Day -2: Same procedures as Day -2 of Part A, except an additional vein assessment.

Day -1:

- All subjects will receive 5 ml of iohexol solution (300 mg/mL iodine) intravenously on Day -1 at the expected time of dosing of study drug DNDi-0690 or placebo on Day 10.
- A brief physical examination
- 12-lead ECG: Pre-iohexol dose
- Vital signs (supine systolic and diastolic blood pressure, pulse rate, and tympanic temperature): Pre-iohexol dose
- AE
- SAE
- Prior and concomitant medication
- Plasma samples for iohexol: Pre-iohexol dose, 1, 2, 3, 4 and 5 h post-iohexol dose
- Urine samples for iohexol: Pre-iohexol dose, [0-1], [1-2], [2-3], [3-4] and [4-5] h post-iohexol dose (240 mL water to be taken after each urine PK sampling timepoint to ensure the capability of the volunteer to provide urine samples during each collection period).

Day 1:

Dose administrations of DNDI-0690 capsule or matching placebo will occur on the morning of Day 1 in a randomised, double-blind manner following an overnight fast of at least 10 hours.

- Eligibility check: Pre-dose
- Troponin I: Pre-morning dose
- 12-lead ECG: Pre-morning dose (in triplicate) and 4 h post-morning dose (single)
- Vital signs (supine systolic and diastolic blood pressure, pulse rate and tympanic temperature): Pre-morning dose
- AE
- SAE
- Prior and concomitant medication
- PK plasma sample for DNDI-0690 and metabolite: Pre-morning dose
- Urine samples for exploratory biomarkers: Pre-morning dose

Day 2:

- Eligibility check: Pre-dose
- Dosing
- Laboratory safety tests (biochemistry, haematology, coagulation, urinalysis, urine microscopy): Pre-morning dose
- AE
- SAE
- Prior and Concomitant Medication
- PK plasma sample for DNDI-0690 and metabolite: Pre-morning dose

- Urine samples for exploratory biomarkers: Pre-morning dose

Day 3, Day 5, Day 6, Day 8 and Day 9:

- Eligibility check: Pre-dose
- Dosing
- AE
- SAE
- Prior and Concomitant Medication

Day 4 and Day 7:

- Eligibility check: Pre-dose
- Dosing
- A brief physical examination
- Laboratory safety tests (biochemistry, haematology, coagulation, urinalysis, urine microscopy): Pre-morning dose
- 12-lead ECG: Pre-morning dose, 4h post-morning dose
- Vital signs (supine systolic and diastolic blood pressure, pulse rate, and tympanic temperature): Pre-morning dose
- AE
- SAE
- Prior and concomitant medication
- PK plasma sample for DNDI-0690 and metabolite: Pre-morning dose
- Urine samples for exploratory biomarkers: Pre-morning dose

Day 10:

On Day 10, DNDI-0690 (6 subjects) or matching placebo (3 subjects) will be administered in the morning only. 5 ml of iohexol solution (300mg/mL iodine) will be administered intravenously immediately after dosing of DNDI-0690.

- Eligibility check: Pre-dose
- Laboratory safety tests (biochemistry, haematology, coagulation, urinalysis, urine microscopy): Pre-dose
- Troponin I: Pre-dose
- 12-lead ECG: Pre-dose and 4 h post-dose
- Vital signs (supine systolic and diastolic blood pressure, pulse rate and tympanic temperature): Pre-dose
- AE
- SAE
- Prior and concomitant medication
- PK plasma sample for DNDI-0690 and metabolite: Pre-dose and, 0.5, 1, 2, 3, 4, 6, and 9 h post-dose
- Plasma samples for iohexol: Pre-iohexol dose, 1, 2, 3, 4 and 5 h post-iohexol dose
- Urine samples for exploratory biomarkers: Pre-dose
- Urine sample for iohexol: Pre-iohexol dose, [0-1], [1-2], [2-3], [3-4] and [4-5]h post-iohexol dose (240 mL water to be taken after each urine PK sampling timepoint to ensure the capability of the volunteer to provide urine samples during each collection period)

Day 11:

- AE
- SAE
- Prior and concomitant medication
- PK plasma sample for DNDI-0690 and metabolite: 24 h post-last dose

Day 12:

- A brief physical examination
- Weight

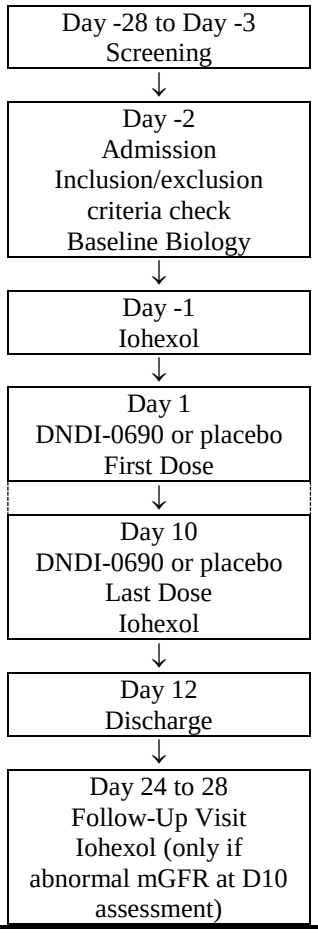
- Laboratory safety tests (biochemistry, haematology, coagulation, urinalysis, urine microscopy)
- 12-lead ECG
- Vital signs (supine systolic and diastolic blood pressure, pulse rate, and tympanic temperature)
- AE
- SAE
- Prior and concomitant medication
- PK plasma sample for DNDI-0690 and metabolite: 48 h post-last dose

If all assessments are satisfactory to the PI (or deputy), subjects will be discharged from clinic after all Day 12 procedures are completed.

Post Study Follow-up (Day 24 to Day 28):

- Post study follow-up procedures are identical to Part A.
- Optional iohexol dosing at the time of the follow-up visit, plasma and urine samples for iohexol may be taken if results from previous timepoints of mGFR calculation showed abnormal results.
  - Plasma samples for iohexol: Pre-iohexol dose, 1, 2, 3, 4 and 5 h post-iohexol dose
  - Urine samples for iohexol: Pre-iohexol dose, [0-1], [1-2], [2-3], [3-4] and [4-5]h post-iohexol dose (240 mL water to be taken after each urine PK sampling timepoint to ensure the capability of the volunteer to provide urine samples during each collection period)

Figure 3 Study Sequence Part C



NUMBER OF SUBJECTS:

Part A: Up to 4 cohorts of 9 subjects

Part B: 9 subjects (Optional)
Part C: 9 subjects

MAIN INCLUSION CRITERIA:

1. Healthy males or healthy women of non-childbearing potential (WONCBP) between 18 and 55 years of age inclusive at the time of signing informed consent
2. Female subject of non-childbearing potential. WONCBP is defined as women who are postmenopausal or permanently sterilised (permanent sterilisation methods include hysterectomy, bilateral salpingectomy, and bilateral oophorectomy).
3. Female post-menopausal state is defined as no menses for 12 months without an alternative medical cause and confirmed by a serum FSH result of ≥40 IU/L at Screening.
4. Male subject (and subject's partner of childbearing potential) must use condom and also willing to use a highly effective method of contraception or 2 effective methods of contraception (see Section 10.5.2), if applicable (unless anatomically sterile or where abstaining from sexual intercourse is in line with the preferred and usual lifestyle of the subject) from first dose until 3 months after last dose of IMP.
5. Body mass index (BMI) of 18.0 to 30.1 kg/m^2 as measured at Screening and body weight ≥60 kg (BMI = body weight (kg) / [height (m)]^2).
6. 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.
7. Subject with a negative urinary drugs of abuse (DOA) screen (including alcohol and 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).
8. Subject with negative human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg)) and hepatitis C virus antibody (HCV Ab) test results at Screening.
9. General good physical health determined by medical and surgical history, physical examination, 12-lead ECG, vital signs and clinical laboratory tests.
10. Normal blood pressure: Systolic blood pressure between ≥90 and ≤140 mmHg, Diastolic blood pressure ≤90 mmHg, measured after 10 min rest in supine position at Screening, admission and pre-dose.
11. A resting heart rate (HR) between ≥50 and ≤100 bpm measured after 10 min rest in supine position at Screening, admission and pre-dose.
12. ECG recording without clinically significant abnormality, including QTcF measure of ≤450 msec at Screening, admission and pre-dose
13. No febrile seizures or infectious illness for at least 7 days prior to first administration of the IMP (Day 1).
14. Must agree to adhere to the contraception requirements and the life-style restrictions.
15. Subject must be available to complete the study (including all follow up visits).
16. Subject must satisfy an Investigator about his/her fitness to participate in the study.
17. Subject must provide written informed consent to participate in the study.

MAIN EXCLUSION CRITERIA:

1. Subjects who have received any IMP in a clinical research study within 90 days prior to Day 1.
2. Subjects who are study site employees, or immediate family members of a study site or sponsor employee.
3. Subjects who have previously been enrolled in this study and/or have received DNDI 0690 previously
4. History of any drug or alcohol abuse in the past 2 years.
5. Demonstrating excess in caffeine/xanthine consumption (more than 6 cups of coffee or equivalent a day).
6. Regular alcohol consumption in males >21 units per week and females >14 units per week (1 unit = ½ pint beer, or a 25 mL shot of 40% spirit, 1.5 to 2 Units = 125 mL glass of wine, depending on type). As confirmed by a positive urine alcohol test at Screening or admission.

7. Current smokers or those who have smoked within the last 12 months with a positive cotinine result at Screening and Admission.
8. Current users of cigarette replacements (*i.e.* e-cigarettes, nicotine patches or gums) and those who have used these products within the last 12 months.
9. Subjects who do not have suitable veins for multiple venepunctures/cannulation as assessed by the Investigator or delegate at Screening.
10. Clinically significant abnormal biochemistry, haematology, coagulation or urinalysis as judged by the Investigator or AST/ALT/total bilirubin/gamma glutamyl transpeptidase [GGT]/ALP/creatinine> 1.2 ULN. Subjects with Gilbert's syndrome are allowed.
11. Positive PCR COVID-19 test at admission.
12. Evidence of renal impairment at Screening or admission, as indicated by an estimated Glomerular Filtration Rate (GFR) <lower limit of normal (LLN) using the CKD-EPI equation.
13. History of clinically significant cardiovascular, renal, hepatic, neurological (especially seizures), immunological, psychiatric, myopathies, bleeding tendency, respiratory and particularly gastrointestinal (GI) disease, especially peptic ulceration and chronic gastritis, GI bleeding, ulcerative colitis, Crohn's Disease or Irritable Bowel Syndrome, as judged by the Investigator.
14. History of additional risk factors for Torsades des Pointe (*i.e.* heart failure, hypokalaemia, family history of long QT syndrome).
15. Rare hereditary problems of fructose intolerance, glucose-galactose malabsorption or sucrose-isomaltase insufficiency.
16. Any relevant GI complaints within 7 days of dosing.
17. Serious adverse reaction or clinically relevant hypersensitivity to any drug or the formulation excipients (Hypromellose [HPMC], sodium lauryl sulphate [SLS], sucrose, croscarmellose sodium and magnesium stearate).
18. Presence or history of clinically significant allergy requiring treatment (including asthma, urticaria, clinically significant allergic rash or other severe allergic diathesis), as judged by the Investigator. Hay fever is allowed unless it is active.
19. Donation or loss of greater than 500 mL of blood within the previous 3 months or more than 100 mL within 30 days prior to signature of informed consent.
20. Subjects who are taking, or have taken, any prescribed or over-the-counter drug (including anti-acid drugs) or vitamins/herbal remedies (*i.e.* St. John's Wort and others which are known to interfere with the CYP3A4 and P-gp metabolic pathways) or hormone replacement therapy (HRT) or any drug known to modify the MATE-1/OCT-2 renal transporters (such as, for example, cimetidine, ritonavir, trimethoprim, cisplatin) in the 28 days before IMP administration (or 5 half-lives of the taken drug, whichever is longer). Administration of up to 4 g of paracetamol per day within 7 days of IMP administration is allowed.
21. Surgery within 12 weeks prior to Screening, with the exception of appendectomy.
22. Any surgery (*i.e.* gastric bypass) or medical condition that may affect absorption of orally administered drugs.
23. Failure to satisfy the Investigator of fitness to participate for any other reason.
24. Breastfeeding and lactating females.

Additional Exclusion Criteria for Part C

25. Hypersensitivity to iohexol or any of its excipients or to iodine, history of allergic reaction to iohexol, iodine or other contrast media, including cutaneous adverse reactions.
26. Manifest thyrotoxicosis.

IMP ADMINISTRATION

Capsules of DNDI-0690 100mg and 200 mg have been chosen as the formulation for this study. The number of capsules to be administered will vary depending on the dose selected by the SRC upon review of data (safety and PK) from the previous cohort.

Table 1 Description of DNDI-0690 doses

Part A	Dose		Increase from the previous dose
	mg	mg/kg/day*	
Cohort 1	400 OD	5.7	
Cohort 2	To be decided	To be decided	2 to 3 times and/or change from OD to BID
Cohort 3	To be decided	To be decided	2 to 3 times and/or change from OD to BID
Cohort 4	To be decided	To be decided	2 to 3 times and/or change from OD to BID
Part B	maximum of 3600	maximum of 51.5	
Part C	To be decided	To be decided	Highest dose tested in Part A and well tolerated, or below

*** assuming a 70-kg person**

Part C
Non-IMP (NIMP): Iohexol solution (300mg/mL iodine). 5 ml of iohexol solution (300mg/mL iodine) will be administered intravenously on Day -1 and Day 10.

Precautions for use as per iohexol summary of product characteristics (SmPC) will be respected[01].

STUDY VARIABLES/ENDPOINTS:

Primary Endpoints

Safety parameters (changes in vital signs, ECG, safety laboratory parameters with special focus on renal function parameters, *i.e.* serum creatinine, blood urea nitrogen (BUN), and serum Cys C), and frequency and severity of observed treatment-emergent adverse events (TEAEs).

Secondary Endpoints

- Measurement of the following PK parameters:
  - Day 1:
    - Main: C_{max}, $C_{max/D}$, AUC_{0-24}, $AUC_{0-24/D}$
    - Exploratory: $AUC_{0-24,norm}$, $C_{max,norm}$, T_{max}, MRT_{last}
  - Day 10:
    - Main: AUC_{0-inf}, AUC_{0-inf}/D, AUC_{0-24}, AUC_{0-24}/D, $C_{max,ss}$, $C_{max,ss}/D$, $C_{min\ SS}$
    - Exploratory: $AUC_{0-inf,norm}$, $AUC_{0-24,norm}$, AUC_{last}, AUC_{last}/D, $AUC_{last,norm}$, $C_{max,ss,norm}$, T_{max}, $t_{½}$, Cl/F, lambda-z, MRT_{last}, Vz/F, CL_{ss}/F and points terminal for DNDI-0690.
- Optional: if BID dosing, AUC_{0-10}, AUC_{0-10}/D, $AUC_{0-10,norm}$ will be added on Day 1 and Day10
- AUC_{t-inf}, $\%AUC_{extra}$
- Accumulation ratios RA(C_{max}) and RA(AUC_{0-tau}) will be calculated
- C_{trough} will be derived from the concentration data (Days 2-10 for Part A, days 2-5 for Part B and days 2/4/7/10 for Part C).
- Measurement of the urine PK parameters Ae, Ae% and CLr for DNDI-0690.
- Glomerular filtration rate measurement (mGFR) following evaluation of plasma clearance of iohexol before and under exposure to DNDI-0690

Exploratory Endpoints

- Changes in clinical early renal toxicity biomarkers in urine: Cystatin C (Cys C), Kidney Injury Molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL).
- Analysis of Holter extracted ECGs for the following parameters: RR, heart rate (HR), PR, QRS, QT, corrected QT interval by Fridericia's formula (QTcF), corrected QT interval by Bazett's formula (QTcB), ΔHR, ΔRR, ΔPR, ΔQRS, ΔQT, ΔQTcF and ΔQTcB.
- Measurement of drug concentration in dry blood spots for comparison with matching plasma samples
- Variation of mRNA expression in blood sample before and after exposure to the drug (Transcriptional Profiling)
- Exploration of metabolism after multiple dose exposure

STATISTICAL METHODS:

Descriptive summaries of baseline, PK and safety data will be presented including changes from baseline as appropriate.

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. Only 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 -2) 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 QTcB and QTcF values will be summarised.

Dose Proportionality/Independence (Part A and Part B)

For Day 1 and Day 10 (Part A) or Day 1 and Day 5 (Part B), dose proportionality will be assessed by performing a regression analysis of the log-transformed C_{max}, $AUC_{0-\tau}$ and $AUC_{0\ inf}$ (Day 10 for Part A and Day 5 for Part B only) 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.

For Day 10 for Part A and Day 5 for Part B, dose independence will be assessed for $t_{½}$ and CL/F by performing a regression analysis of the untransformed parameters versus dose with a fixed effect for dose. For each parameter a point estimate and corresponding 95 % CI will be calculated for the slope of the regression line.

Steady State (Part A and Part B)

For each dose level, log-transformed trough concentration levels at pre-dose each day (Day 2 to Day 10 for Part A and Day 2 to Day 5 for Part B) will be subjected to a mixed effects analysis of variance (ANOVA) with study day as a fixed effect and subject as a random effect in order to establish whether and when steady state has been

attained for each dose level. Back-transformed ratios for the comparisons of each consecutive day (*i.e.* Day 3/Day 2) will be presented along with corresponding 90 % CI.

Accumulation (Part A and Part B)

For each dose level, log-transformed C_{max} and $AUC_{0-\tau}$ values on Day 1 and Day 10 for Part A or Day 1 to Day 5 for Part B will be subjected to an ANOVA with study day as a fixed effect and subject as a random effect. For comparison point estimates and 90 % CI for the difference between Day 10 (Part A) or Day 5 (Part B) and Day 1 will be constructed using the residual mean square error obtained from the ANOVA for each dose level. The point and interval estimates will then be back transformed to give estimates of the ratios of the geometric least squares means and corresponding 90 % CI.

Measured glomerular filtration rate (mGFR) will be calculated following evaluation of plasma clearance of iohexol before and under exposure to DNDI-0690. mGFR data will be descriptively analysed together with their 95% confidence intervals.

Details on the statistical analyses will be presented in the statistical analysis plan (SAP), which will be finalized and signed before unblinding the study.

DURATION OF STUDY:	Screening will occur within 28 days before admission to the research unit prior to DNDI-0690 dosing. Multiple oral administration per subject OD or BID for 10 days (Part A and Part C) or OD for 5 days (Part B). Duration of clinical phase by subject will be approximately 2 months.

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FIGURES

5 ABBREVIATIONS USED IN THE PROTOCOL TEXT

Abbreviation	Definition
ABPI	The Association of the British Pharmaceutical Industry
ADR	adverse drug reaction
AE	adverse event
ALB	Albumin
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AmpB	amphotericin B
ANOVA	analysis of variance
API	The active pharmaceutical ingredient
aPTT	activated partial thromboplastin time
AST	aspartate aminotransferase
AUC	area under the concentration versus time curve
AUC_{0-24}	AUC from time zero to time 24 h post-dose
AUC_{0-inf}	AUC extrapolated to infinity from dosing time, based on the last measurable concentration
AUC_{0-last}	AUC from the time of dosing to the time of the last measurable concentration
$AUC_{0-\tau}$	AUC from time of dosing to the end of the dosing interval
BIA	The BioIndustry Association
BID	twice a day
BMI	body mass index
BUN	blood urea nitrogen
CCRA	The Clinical Contract Research Association
CL	cutaneous leishmaniasis
CNS	central nervous system
COVID-19	coronavirus disease 2019
C_{max}	maximum concentration
$C_{max, norm}$	maximum concentration divided by dose per kilogram body weight
$C_{max, SS}$	maximum concentration at steady state
$C_{min, SS}$	minimum concentration at steady state
CRF	case report form
eCRF	electronic case report form
CV%	coefficient of variation
CYP	Cytochrome P450
Cys C	Cystatin C
DDI	drug-drug interaction
DMP	data management plan
DOA	drugs of abuse
EC	Ethics Committee
ECG	electrocardiogram
EDTA	ethylenediaminetetraacetic acid
EMA	European Medicines Agency
Fe (%)	% mean cumulative urine

FDA	US Food and Drug Administration
FIH	First-in-Human
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GFR	glomerular filtration rate
GFRcc	glomerular filtration rate based on serum cystatin C levels
GFRe	glomerular filtration rate based on serum creatinine levels
mGFR	measured glomerular filtration rate
GGT	gamma glutamyl transferase
GI	gastrointestinal
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GP	general practitioner
HBsAg	hepatitis B surface antigen
HCV Ab	hepatitis C virus antibody
HDPE	high density polyethylene
HED	human equivalent dose
hERG	the human Ether-à-go-go-Related Gene
HIV	human immunodeficiency virus
HPMC	hydroxypropyl methylcellulose
HR	heart rate
HRA	Health Research Authority
HRT	hormone replacement therapy
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonisation
IMP	investigational medicinal product
INR	international normalised ratio
ISF	investigator site file
IUD	intrauterine device
IUS	intrauterine hormone-releasing system
KIM-1	kidney injury molecule-1
LLN	lower limit of normal
LLOQ	the lower limit of quantitation
LSP	laboratory services plan
MAD	multiple ascending dose
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
NCE	New Chemical Entity
NGAL	neutrophil gelatinase-associated lipocalin
NOAEL	no observed adverse effect level
OD	once daily

PCH	Prince Charles Hospital
PCR	polymerase chain reaction
PD	pharmacodynamics
PEG	polyethylene glycol
PI	principal investigator
PIS	participant information sheet
PK	pharmacokinetic(s)
PPE	personal protective equipment
PT	prothrombin time
PV	pharmacovigilance
PWG	Pathology Working Group
QA	quality assurance
QC	quality control
QD	one a day
QP	Qualified Person
QTcB	corrected QT interval by Bazett's formula
QTcF	corrected QT interval by Fridericia's formula
RBC	red blood cells
RDW	red blood cell distribution width
REC	Research Ethics Committee
SAD	single ascending dose
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
SHM	sample handling manual
SLS	sodium lauryl sulfate
SMP	safety management plan
SmPC	Summary of Product Characteristics
SOC	system organ class
SOP	standard operating procedure
SRC	safety review committee
SUSAR	suspected unexpected serious adverse reaction
TDI	time-dependent inhibition
TEAE	treatment-emergent adverse event
TMF	trial master file
eTMF	electronic trial master file
T_{max}	time to reach maximum concentration
ULN	upper limit of normal
VL	visceral leishmaniasis
WBC	white blood cells
WHO	World Health Organisation
WONCBP	women (woman) of non-childbearing potential

6 ETHICS

6.1 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 confirmation of an appropriate deferral from the Health Research Authority (HRA) if applicable.

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 AEs it will not be recommenced without reference to the REC responsible for the study.

The outcome of the study (*i.e*. completed) will be reported to the REC responsible for the study within 90 days of completion of the last subject'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 subject's final study procedures.

The REC will be informed that Simbec-Orion is a commercial organisation and that the study is funded by DNDi. The subjects 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.

6.2 Ethical Conduct of the Study

The 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 Conference on Harmonisation (ICH) Good Clinical Practice (GCP)[03] and applicable regulatory requirements.

6.3 Participant Information and Consent

Potential subjects 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 subject 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 (PIS)) and informed consent form (ICF) will be provided, and the subject will be allowed sufficient time to consider the study information. Prior to signing the ICF, the subject 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 subject is willing to participate in the study, the ICF will be signed and personally dated by the subject and the person taking consent. The subject will receive a copy of the ICF together with the PIS and the original signed informed consent form 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 subject's medical record (*i.e.* source document).

7 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 Ezanul Wahab. The Project Manager will act on behalf of the PI to ensure the smooth and efficient running of all aspects of the study.

7.1 Study Personnel

Contract Research Organisation: Simbec-Orion, United Kingdom

- Principal Investigator: Ezanul Wahab
- Project Manager (Main Contact): Lan Tann
- Project Manager Deputy: Matilda Greenstreet
- Pharmacokinetics (PK): Simon Hutchings
- Statistics: Virginie Combe
- 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
- Sponsor's Medical Responsible / Responsible Physician: Henri Caplain

Monitor: Simbec-Orion Clinical Development, United Kingdom

Pharmacovigilance (PV):

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

Bio-analytics: SGS LIFE SCIENCES, Belgium

Central safety ECG reading and extraction of ECGs from Holter recordings: Banook Group, France

7.2 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 and the National Research Ethics Service[04].

8 INTRODUCTION

Leishmaniasis is generally seen as one of the most neglected tropical diseases which has strong links with poverty. It comprises a complex vector-borne disease, caused by more than 20 species of the protozoan genus Leishmania and 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.

Much progress has been made in VL treatments over the last 15 years, moving from the sole antimonial monotherapy to the development of new treatments including liposomal formulations of amphotericin B (AmpB), the oral drug miltefosine and injectable paromomycin. However, these drugs still bear limitations such as long treatment duration, parenteral administration, poor safety profile or need for cold chain. There is a great need for an innovative therapy that is efficacious, safe and can be deployed in remote areas where VL occurs, ideally with pan-geographical efficacy. In addition, the new therapies have to be adapted to children, as they represent more than 50% of VL patients worldwide

Antimonials continues being 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, 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%.

DND*i*'s priority is to eliminate the use of antimonials and develop at least one safe, effective, oral, short-course VL/CL drug to replace current treatments that can be used at any healthcare level in all foci of the disease.

DNDi has developed DNDI-0690, a new chemical entity (NCE) which has demonstrated excellent efficacy in animal models of *Leishmania* infection. It is anticipated that DNDI-0690 could provide a shorter treatment course by the oral route, as well as an improved therapeutic index, therefore making it a promising NCE to develop for VL and for CL.

This study is **not** the first to administer DNDI-0690 to humans.

8.1 Physical, Chemical and Pharmaceutical Properties and Formulation

The active pharmaceutical ingredient (API) is DNDI-0690, a 7-substituted nitroimidazooxazine compound.

The investigational product will be supplied as hard gelatin capsules in high density polyethylene (HDPE) bottles with induction seal closures. Each capsule contains either 100 mg or 200 mg of DNDI-0690. Each dose strength is presented as a Swedish orange, size 00 capsule. The investigational product also contains the following non-active ingredients: hydroxypropyl methylcellulose (HPMC), sodium lauryl sulfate (SLS), sucrose, croscarmellose sodium and magnesium stearate.

The matching placebo is made of the same non-active excipients as the investigational product (sucrose, croscarmellose sodium and magnesium stearate).

DNDI-0690 capsules (active and placebo) should be stored in the original container and not above 30°C.

8.2 Nonclinical Pharmacology

8.2.1 Efficacy: *in vitro* activity

DNDI-0690 is a 7-substituted nitroimidazooxazine, which showed a very potent antileishmanial activity and demonstrated broad spectrum *in vitro* activity against a range of *Leishmania* strains (see Table 2), comparing favorably with the standard antileishmanial drugs SSG and miltefosine. Des-Nitro analogues of DNDI-0690 have been shown to be inactive against *Leishmania in vitro*, confirming that the nitro (NO_2) group is essential for DNDI-0690 cidal activity. Activation via the parasite nitroreductase 2 seems to be required. However, the mechanism of action of DNDI-0690 for antileishmanial activity still remains to be elucidated.

Table 2 Summary of in vitro Primary Pharmacodynamic Activity of DNDI-0690 (Activity Against *Leishmania donovani* Strains)***:**

	Strain ID	**Strain origin**	**DNDI-0690**
			IC_{50} values (µM)
L. donovani	MHOM/IN/80/DD8	India WHO reference strain	**0.72**
	MHOM/ET/67/HU3	Ethiopia WHO reference strain	**0.03**
	BHU1	India, clinical isolate	**2.50**
	SUKA001	Sudan, clinical isolate	**0.68**
	GR265	Ethiopia, clinical isolate	**0.91**

** methodology, additional L. strains and comparison with other drugs are available in the IB*

8.2.2 *in vivo* Efficacy and Human Dose Prediction

DNDI-0690 administered by oral route is efficacious in mouse and hamster models of both acute and chronic VL infection (by *L. infantum* and *L. donovani*, see IB for details). DNDI-0690 also showed efficacy in CL murine models.

Unbound cumulative plasma exposure ($AUC_{cum,u}$) showed to be the best predictor of efficacy, for the murine as well as the hamster VL models. In order to reach the targeted parasite burden reduction of 95% compared to vehicle treated groups (ED95), an unbound cumulative AUC of 0.48 µg/mL*h was sufficient in the hamsters when infected by either *L. infantum* or *L. donovani*. Higher and different free cumulative AUC values were required for the mouse model where 1.4 µg/mL*h and 4.0 µg/mL*h gave 95% efficacy following an infection with *L. infantum* and *L. donovani*, respectively.

Therefore, and based on a conservative approach, a range of unbound cumulative AUC from 0.48 µg/mL*h to 4.0 µg/mL*h has been determined to be potentially effective in humans.

8.3 Safety Pharmacology

Safety pharmacology studies have shown that DNDI-0690 had no significant effects on central nervous system (CNS), respiratory function and the cardiovascular system. In particular, no effects on general behavior and body temperature were observed in rats up to 500 mg/kg BID.

In addition, DNDI-0690 did not show any effect on the respiratory function in rats up to the dose of 500 mg/kg one a day (QD). In the human Ether-à-go-go-Related Gene (hERG) assay, an inhibition of the potassium channel was observed at concentrations (IC_{50} = 10.29 µM) much higher than those showing efficacy against *Leishmania* strains *in vitro* (23-368 nM as free fraction). The absence of any effect on the cardiovascular system was confirmed *in vivo* in a telemetry study in monkeys, where no effects on cardiovascular parameters and body temperature were seen up to the single dose of 350 mg/kg.

8.4 Nonclinical Pharmacokinetics and Product Metabolism

DNDI-0690 appeared to be very slowly metabolized in vitro and in vivo, leading to a consistent metabolic pattern across species. Only mono-oxygenation at very small or negligible relative peak areas compared to parent was detected in rat, dog, cynomolgus and human microsomes. The same circulating metabolite was observed in rat plasma, while it was combined with nitro-reduction in cynomolgus plasma. In all cases, the circulating metabolites detected in vivo in rats and monkeys represented minor levels. No metabolite needed follow-up quantitation in toxicology species. As a consequence, the metabolic profile of DNDI-0690 is unlikely to be different in human since no human specific metabolite was identified in vitro. All human metabolites detected so far in vitro are covered by toxicology species used as part of the regulatory preclinical package (in rat and monkey).

The pharmacokinetic profile of DNDI-0690 in rat, dog and monkey is characterized by a low to moderate plasma clearance and a moderate half-life related. Volume of distribution in all species is approximately equal to the volume of body water.

In the single ascending dose (SAD) study, at doses ranging from 10 mg to 3600 mg, DNDI-0690 concentrations declined in a monophasic or biphasic manner with resultant geometric mean half-life ranging from 4.547 to 12.144 h across all regimens with no impact of food or gender. Results from the SAD study were used to predict human doses at steady state.

Fraction excreted in human urine in the SAD was lower than expected with a mean cumulative urine Fe (%) values over 72 hours of between 0.00350% and 0.0220% across all groups evaluated.

Based on current drug-drug interaction (DDI) risk assessment strategies, there appears to be no perpetrator DDI risk for DNDI-0690 due to potential cytochrome P450 (CYP) inhibition (including time-dependent inhibition (TDI)), CYP induction as well as inhibition of main relevant transporters. One single drug transporter, MATE1, was inhibited at 55% in presence of 10 µM of DNDI-0690. This data suggests that DDI is possible if DNDI-0690 is co-administrated with a drug mainly eliminated through MATE1 transport.

8.5 Toxicology

8.5.1 Regulatory Toxicology Studies

The non-clinical safety of DNDI-0690 was assessed in repeated dose toxicity studies in rats and monkeys following oral administration of the compound daily for up to 4 weeks.

In rats, a first GLP 14-day toxicity study was conducted with DNDI-0690 formulated in polyethylene glycol (PEG) 400 and administered as single daily doses up to 250 mg/kg/day. In

this study, the NOAEL was set at 100 mg/kg/day (AUC_{24} on Day 14 of 93.6 and 149 µg·h/mL in males and females, respectively) since some histopathological changes in the liver (hepatocellular hypertrophy) and adrenals (cortical vacuolation and hypertrophy) were detected at the dose of 250 mg/kg/day.

Subsequent toxicity studies both in rats and in monkeys were performed using DNDI-0690 formulated as nanosuspension.

In the GLP 4-week repeated dose toxicity study in rats, only minimal reduction of body weight and food consumption, with concomitant slight decrease in globulin and total cholesterol, were seen at doses of 200 and 1000 mg/kg/day (100 and 500 mg/kg BID 6-h apart). No other effects were evidenced. Based on these findings, the dose of 40 mg/kg/day was the NOEL and the dose of 1000 mg/kg/day was the NOAEL.

In the GLP 4-week toxicity study in monkeys, doses of 20, 100 and 400 mg/kg/day (10, 50, 200mg/kg BID 6-h apart) were selected. One female died at the dose of 100 mg/kg/day and another at 400 mg/kg/day on Days 23 and 29, respectively. Deaths were related to severe effects on the heart and the kidneys, the animals were severely dehydrated, and the adverse effects on the heart being likely a consequence of renal toxicity. In other monkeys treated at these dose levels, noteworthy effects of DNDI-0690 included renal effects in females and histological changes in the testes in males (see below). Changes in the kidneys were characterized by tubular dilatation, cell degeneration and/or basophilia, associated with increases in creatinine and urea. Kidney changes completely recovered, as evidenced at the end of a 7-week recovery period. In this study, no effects were observed at the dose of 20 mg/kg/day that was considered as the NOAEL in monkeys after 4-week administration.

A Pathology Working Group (PWG) was constituted to review the data on testes. For the 7–day study, the PWG concluded that the testicular effect of vacuolation was a background change and that the effects seen were all considered as fixation artefact and thus the testicular histology was all concluded to be within the normal. In the case of the 28-day study, the PWG confirmed following review the presence of changes in the testes of animals at the two highest doses of 100 and 400 mg/kg/day (the same animals as in the initial report) where the finding was described by the PWG as segmental tubular dilation with associated thinning/atrophy of the seminiferous epithelium. This distribution could suggest a potential relationship to treatment. However, based upon the segmental nature of the finding, the frequent unilateral distribution and the fact that this finding has been described in a number of publications as a background change in young cynomolgus monkeys, it was concluded that the findings in the testes were a background lesion and unrelated to treatment. In conclusion, treatment with DNDI-0690 for 7 or 28 days is not considered to induce any testicular changes or toxicity.

Table 3 Summary of Toxicokinetic and Pharmacokinetic Parameters for DNDI-0690*

Species / Day	Gender	Type of study	Dose PO (mg/kg/day)	C_{max} (µg/mL)	AUC_{0-24} (µg.h/mL)
Rat / Day 28	M	Toxicology TK	1000 (500 bid) NOAEL 28 days	11.3 ± 0.693	180 ± 22.5
	F	Toxicology TK	1000 (500 bid) NOAEL 28 days	16.9 ± 1.47	307 ± 36.8
Monkey / Day 28	M	Toxicology TK	20 (10 bid) NOAEL 28 days	8.03 ± 0.367	101 ± 7.85
	F	Toxicology TK	20 (10 bid) NOAEL 28 days	9.03 ± 6.78	111 ± 61.2

** methodology, additional doses and time-points available in the IB*

In conclusion, preclinical toxicology results indicate that the main target for toxicity of DNDI-0690 is the kidney. Potential renal toxicity can be closely monitored in clinical trials through standard laboratory examinations and specific renal parameters (creatinine, BUN, Cys C).

Due to heart changes as a consequence of nephrotoxicity were considered agonal in their nature and contributory to death together with renal changes, circulating levels of troponin I will continue to be monitored in the multiple ascending dose (MAD) study in healthy subjects at admission, at last day of treatment and in case of any ECG clinically significant abnormality during the course of treatment.

8.5.2 Phototoxicity

DNDI-0690 does not absorb light within the range of natural sunlight (290-700 nm –λmax 255 nm), therefore no phototoxicity is expected.

8.5.3 Genotoxicity

Standard in vitro and in vivo genotoxicity testing did not suggest any genotoxic/clastogenic potential for DNDI-0690. Nitro- reduction was also investigated, but no effect was detected in GLP genotoxicity studies (with activation by S9 fraction). DNDI-0690 is therefore not expected to pose a genotoxic concern for humans.

8.6 Summary of Clinical Data

8.6.1 Single Ascending Dose Results in Healthy Volunteers

DNDI-0690 in capsule form was well tolerated in the fasted state at dose levels 10, 30, 400, 1200 and 3600 mg in male healthy subjects, and 1200 mg in female subjects. In the fed state, 400 mg DNDI-0690 was well tolerated in male subjects.

- There were no SAEs, but 18 non-serious TEAEs were reported.
- In the active IMP group, the most common TEAEs were headache and flatulence. Headache was reported by 3 male subjects (8.3% of total fasted male subjects under

DNDI-0690), followed by flatulence reported by 2 male subjects (5.6 % of total fasted male subjects under DNDI-0690). Flatulence was reported by 2 female subjects (33.3% of total fasted female subjects under DNDI-0690). No other TEAEs were reported by more than 1 subject.

- Overall, TEAEs were reported in a similar proportion of subjects receiving DNDI-0690 and matching placebo. The most common TEAEs were headache and flatulence, assessed as mild in severity and which resolved spontaneously.

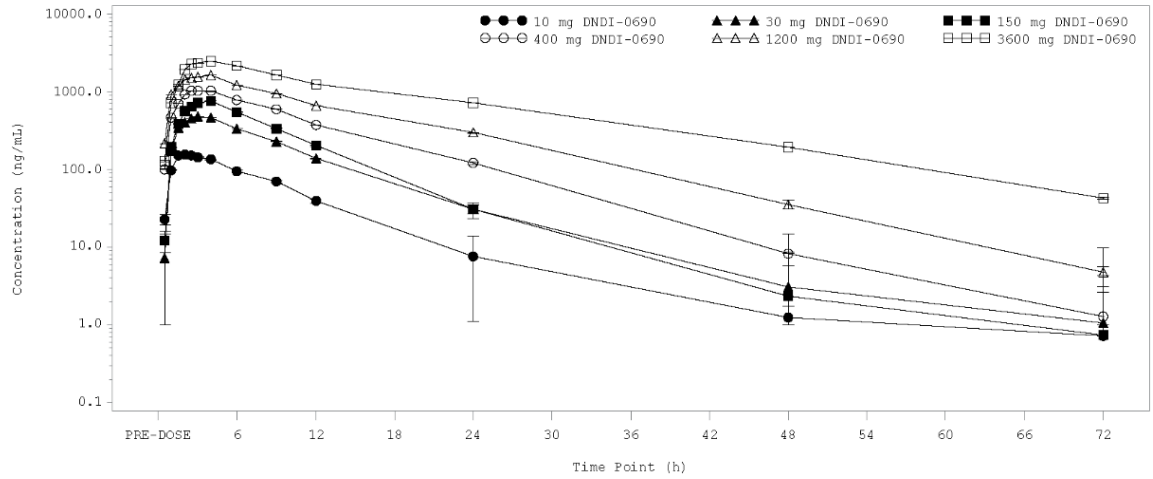
There were no clinically significant findings in laboratory assessments, including Cys C, urea, BUN, troponin I, AST, ALT, bilirubin, cholesterol, urinalysis, vital signs, physical examinations or body weight. A slight increase was observed in creatinine in all cohorts however, this was not of clinical significance. mGFR (based on urine creatinine concentration, urine volume and plasma creatinine level) and estimated based on creatinine levels (GFRe) showed an apparent reduction of 12-14%, however, no change in Cys C levels or GFR estimated based on Cys C levels (GFRcc) was observed.

There were no clinically significant findings in ECG results and no notable increases in QTcF interval for any treatment dose.

There were no notable dose related trends in safety results and no major differences between treatments. The maximum dose administered in the study was 3600 mg single dose (18 capsules of 200mg). Further dose-escalation was not considered for practical reasons, *i.e.* the number of capsules administered could not be increased, and because the plasma exposure predicted for efficacy was reached. However, no limiting toxicity was observed at this dose and DNDI-0690 was considered safe and well tolerated up to 3600 mg single dose.

Following single oral administration of DNDI-0690 in capsule form in the fasted state over the 10 mg to 3600 mg dose range, exposure to DNDI-0690 increased in a sub-proportional manner based on C_{max}, AUC_{last} and $AUC_{0\text{-}inf}$.

Figure 4 **Geometric Mean (×/÷ Geometric SD) Plasma DNDI-0690 Concentrations (ng/mL) Following Single Oral Doses of 10, 30, 150, 400, 1200 and 3600 mg in Healthy Males in the Fasted State: Pharmacokinetic Population**



Note: Data in the above graph are presented in Table 14.2.1.1.1
All subjects in this analysis received DNDI-0690 as a single oral dose
Concentrations reported as BLOQ have been set to 1/2 LLOQ (LLOQ = 1 ng/mL)
except pre-dose which have not been plotted

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Peak and overall exposure levels of DNDI-0690 were increased (between 67% and 75%) in the fed state (400 mg dose level) when compared to the fasted state. These increases were statistically significant for C_{max} but not for AUC due to the higher between subject variability seen in the overall exposure levels.

Plasma exposure (C_{max} and AUC) of DNDI-0690 was statistically significantly increased when dosed in female subjects compared with male subjects (at same 1200 mg dose level). When normalized by weight, plasma exposure was still higher in female subjects than in male subjects, but the difference was no longer statistically significant (between 12% and 33% higher on average).

Following single oral administration of DNDI-0690 in capsule form at doses ranging from 10 mg to 3600 mg, DNDI-0690 was absorbed with median T_{max} occurring between 1.500 h and 4.000 h post-dose with no impact of food or gender.

Over the 10 to 3600 mg range, DNDI-0690 concentrations declined in a monophasic or biphasic manner with resultant geometric mean half-life ranging from 4.547 to 12.144 h across all regimens, with no impact of food or gender. The proportion of DNDI-0690 eliminated via renal route was low across all regimen with mean cumulative urine Fe (%) values over 72 hours of between 0.00350% and 0.0220%.

The inter-subject variability associated with exposure was low to moderate over the 10 mg to 3600 mg dose range with no impact of food, however, variability appeared lower in females compared to males.

At the highest dose level of 3600 mg DNDI-0690, the maximum values of C_{max} and AUC_{0-24} in individual subjects were 3160 ng/mL and 42,600 ng.h/mL respectively. These values were

60.5% and 57.4% lower, respectively, than the maximum exposure cap as defined in the protocol (C_{max} ≥8000 ng/mL or AUC_{0-24} ≥100,000 ng.h/mL).

8.6.2 Multiple Dose Regimen

The present trial aims at evaluating the safety, tolerability, and the PK parameters of DNDI-0690 in healthy volunteers after multiple dose administration in the fasted state. This will bring crucial information to evaluate if further development of this compound will be possible in patients affected by leishmaniasis.

8.7 Risk/Benefit Assessment

No benefit will be observed in healthy volunteers.

The risk/benefit ratio of the proposed study is considered acceptable. Several measures to minimize the risks to healthy subjects have been taken with respect to the following study design elements:

- For each cohort in Part A and Part B, 2 subjects (1 subject on active drug and a subject on matching placebo) will be dosed and monitored for 48 hours prior to dosing the rest of the cohort.
- Dose escalation (during Part A and between Part A and Part B) will be done in a stepwise, controlled manner, with dose escalation only being permitted after full evaluation of the available safety data from the previous cohort by the SRC.
- Clinical laboratory monitoring: At time of study entry, study participants are required to have safety laboratory values within acceptable ranges. Serial measurements of safety laboratory parameters (Biochemistry and haematology which include platelet count, renal/liver function tests, renal integrity monitoring) and coagulation parameters are planned with interim reviews as defined in the SRC charter.
- Close cardiac safety monitoring by regular ECG assessments.
- Study eligibility: The healthy subject/patient inclusion and exclusion criteria have been incorporated to minimize any risk of an exaggerated pharmacological effect of DNDI-0690.
- The evaluation of GFR by a reference method in Part C will be performed at the end of the trial. The dose level used in Part C will be tested in Part A and will have no impact on the safety of the volunteers.
- The starting dose in Part A of this study is 400 mg once daily for 10 days which is considered to be safe and is well below the highest dose level of 3600 mg DNDI-0690 administered in the SAD study. It is expected that the maximum values of C_{max} and AUC_{0-24} in individual subjects in Part A Cohort 1 will be well below the PK stopping criteria of C_{max} ≥8000 ng/mL or AUC_{0-24} ≥100,000 ng.h/mL.
- Main risk associated with iohexol use is anaphylactic reaction. Hypersensitivity reactions may occur irrespective of the dose and mode of administration and mild symptoms may represent the first signs of a serious anaphylactoid reaction/shock. Administration of the contrast medium must be discontinued immediately and, if necessary, specific therapy instituted via the vascular access. Volunteers known to be hypersensitive to iohexol and/or iodine or with history of allergy will be excluded from

the trial. Emergency care and resuscitation are available at the study site in order to provide immediate care to the volunteer if a hypersensitivity reaction was to occur.

- Dose of iohexol planned within the current protocol is very small compared to the dose used in clinic as an intra-vascular administered contrast agent. Common adverse events expected for this product are feeling hot, transient changes in respiratory rate, respiratory distress. Uncommon adverse effects are feeling cold, hyperhidrosis, vasovagal reactions, pain and discomfort at injection site. Rare adverse events are headache, vomiting, diarrhoea, dizziness, hypersensitivity (see above), paresis, paralysis, photophobia, somnolence, arrhythmia (including bradycardia, tachycardia), visual impairment, pyrexia, cough, respiratory arrest, rash, pruritus, urticaria, asthenic conditions (including malaise, fatigue), impairment of renal function including acute renal failure. Hydration is ensured by regular fluid intake on the days of iohexol dosing to minimize the risk to the kidney.
- Other adverse effects of the product are even less frequent, and thus unlikely to pose an important risk to the healthy volunteers.

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

This study is to be conducted in healthy adult volunteers who are deemed not to 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/; last updated 11 September 2020).

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, volunteers 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. When 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.

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 SAEs during the COVID-19 pandemic (details contained within Simbec-Orion Clinical General Risk Assessment_v1.0 (16 June 2020)).

The IMP for this study is DNDi-0690 an oral capsule developed for the treatment of Leishmaniasis. There is no scientific evidence regarding DNDI-0690's impact on a subject's susceptibility to COVID -19 or a subject's condition should they contract COVID-19. 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.

Further details of the non-clinical studies and a summary of the known and potential risks and benefits to human subjects of DNDi-0690 can be found in the IB[05].

The study will be conducted in compliance with the protocol, GCP and the applicable regulatory requirement(s), as indicated within Section 6.2.5 of the ICH GCP E6 (R2) guidelines[03].

9 STUDY OBJECTIVES

9.1 Part A & B

Primary Objective: To assess the safety and tolerability of DNDI-0690 after multiple oral doses in healthy subjects in fasted conditions.

Secondary Objective: To investigate plasma and urinary pharmacokinetics (PK) of DNDI-0690 after multiple oral doses in healthy subjects in fasted conditions.

Exploratory Objectives

- To investigate any potential changes to renal toxicity markers in urine.
- To assess the effect of DNDI-0690 on Holter electrocardiogram (ECG) parameters.
- To compare PK bioanalysis in fixed volume dry blood spots versus PK bioanalysis in plasma samples.
- To investigate the metabolite profile of DNDI-0690 after multiple dosing.
- To assess variation of mRNA expression in full blood before and after exposure to the drug (Transcriptional Profiling).

9.2 Part C

Primary Objective: To evaluate renal function (glomerular filtration rate (GFR)) before and after administration of DNDI-0690 in healthy subjects in fasted condition at maximum well tolerated dose tested in Part A or below.

10 INVESTIGATIONAL PLAN

10.1 Overall Study Design and Plan

The study will be conducted in three parts (Part A, Part B and Part C).

- **Part A** of the study is a single centre, double-blind, randomised, placebo-controlled, parallel-group, multiple oral dose, dose-escalation study to assess the safety, tolerability and PK of DNDI-0690 after multiple oral doses in the fasted condition in healthy male and woman of non-childbearing potential (WONCBP) subjects.
    - Part A will consist of up to 4 cohorts of 9 subjects. Subjects will be randomly assigned to receive an oral dose of active IMP (6 subjects) or matching placebo (3 subjects) for 10 days in a sequential escalating manner with a minimum of 7 days interval between two cohorts.
    - In Part A, each cohort will follow a sentinel dose-escalation schedule according to European Medicine Agency (EMA) guideline[11]; two (2) subjects will be dosed on the first dosing day of EACH cohort (1 subject on active IMP and 1 subject on matching placebo). The remainder of the cohort (5 subjects on active IMP and 2 subjects on matching placebo) will be dosed a minimum of 48 hours later pending confirmation of an acceptable safety profile in the dose-leader cohort by the PI, or medically-qualified designees who are familiar with the study protocol and IB.
- **Part B** of the study is a single centre, double-blind, randomised, placebo-controlled, multiple oral dose study to assess the pharmacodynamic effect of DNDI-0690 on cardiac function after multiple oral doses at the supra-therapeutic dose in fasted condition in healthy male and WONCBP subjects. Part B is optional.
    - Part B will consist of 1 cohort of 9 subjects. Subjects will be randomly assigned to receive an oral dose of active IMP (6 subjects) or matching placebo (3 subjects) for 5 days. Part B will only be implemented if the SRC considers it safe and appropriate to proceed to dose-escalation (with a maximum considered dose of 3600 mg).
    - Subjects in Part B will follow a sentinel schedule; two (2) subjects will be dosed on the first dosing day (1 subject on active IMP and 1 subject on matching placebo). The remainder of the cohort (5 subjects on active IMP and 2 subjects on matching placebo) will be dosed at least 48 h later pending confirmation of an acceptable safety profile in the dose-leader cohort by the PI, or medically-qualified designees who are familiar with the study protocol and IB.
- **Part C** of the study is a single centre, double-blind, randomised, placebo-controlled, multiple oral dose study to evaluate the renal function (mGFR) before and after multiple oral doses in fasted condition in healthy male and WONCBP subjects.
    - Part C will consist of 1 cohort of 9 subjects.
    - Subjects will be administered DNDI-0690 or placebo once daily for 10 days at a dose level that is either at or below the highest well tolerated dose of DNDI-0690 as evaluated in the Part A dose escalating cohorts. The dose will be

decided by the SRC after reviewing data from Part A. As this dose will already have been explored, no sentinel group will be implemented in this cohort.

- All subjects will receive 5 mL of iohexol solution (300 mg/mL iodine) intravenously on Day -1, Day 10, and optionally Day 24-28 that will be flushed with 10 mL of normal saline solution. On Day -1, iohexol will be administered at the same time as expected time of dosing of study drug on Day 10. On Day 10, iohexol will be administered immediately after dosing of study drug.

The clinical phase is anticipated to take place between November 2020 and May 2021. The conclusion of the study is defined as last subject 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 6, Table 7 and Table 8.

10.2 Dose Escalation Procedures and Stopping Criteria

10.2.1 Dose Escalation Procedures (Part A and Part B)

Following completion of each cohort in Part A, a summary of all available safety (AE, 12-lead ECG, vital signs and safety laboratory data) and 24 hour post last dose PK data (minimally C_{max} and AUC_{0-24} data) will be produced on behalf of the PI. Planned doses may be modified following a review of emerging data. Progression to the next dose level and dose selection will be based on the available safety and PK data from all evaluable 9 subjects from the preceding dose level (a subject 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 C_{max} and AUC_{0-24} irrespective of whether they have received active or placebo treatment). 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, an intermediate dose or a lower dose may be given following discussion between the Sponsor and the PI (or deputy).

There will be a Telephone Conference at a pre-appointed time to involve the Simbec-Orion Project Manager and the PI (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.

Part B dosing regimen will be decided after reviewing all available Part A safety and PK data following the same rules and procedures as for Part A.

Dose escalation stopping criteria are detailed in Section 10.2.2.

10.2.2 Stopping Criteria

10.2.2.1 General Stopping Criteria

The study will be discontinued if any unacceptable safety findings are identified. This decision will be made jointly by the PI (or deputy) and the Sponsor. A written document signed by the PI (or deputy) and Sponsor will be produced ratifying the decision.

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

10.2.2.2 Dose Stopping Criteria Within a Cohort (Part A, Part B and Part C)

Dosing of subjects in the ongoing cohort will be temporarily stopped pending evaluation of all available data if any of the following criteria are fulfilled:

- A serious adverse reaction (*i.e.* a SAE considered at least possibly related to the IMP administration) occurs in one subject.

Or

- Severe non-serious adverse reactions (*i.e.* severe non-serious AE considered at least possibly related to the IMP administration) occur in two subjects in the same cohort, independent of within or not within the same system organ class.

Or

- If two or more subjects in the dose group experience any of the following:
  - ALT or AST >3xULN, considered clinically significant and IMP related.
  - Serum Cys C increase > 25%, confirmed after repeat assessment 24-48h apart, and considered IMP related
  - Ratio of microalbuminuria/creatininuria > 300 mg/g and considered IMP related
  - an increase in QTcF value of >60 msec from baseline (average of baseline triplicate values) or an absolute QTcF >500 msec, both confirmed by repeat measurement after 20 minutes in resting position, and considered IMP related.

10.2.2.3 Dose Escalation Stopping Criteria

Dose escalation between cohorts in Part A (and between Part A and Part B) will be temporarily stopped pending evaluation of all available data if any of the following criteria are fulfilled:

- A serious adverse reaction (*i.e.* a SAE considered at least possibly related to the IMP administration) occurs in one subject.

Or

- Severe non-serious adverse reactions (*i.e.*, severe non-serious AE considered as, at least possibly related to the IMP administration) occur in two subjects in the same cohort, independent of within or not within the same system organ class.

Or

- If two or more subjects in the preceding dose group experience any of the following:

- ALT or AST >3xULN, considered clinically significant and IMP related.
- Serum Cys C increase > 25%, confirmed after repeat assessment 24-48h apart, and considered IMP related.
- Ratio of microalbuminuria/creatininuria >300 mg/g and considered IMP related.
- an increase in QTcF value of >60 msec from baseline (average of baseline triplicate values) or an absolute QTcF >500 msec, both confirmed by repeat measurement after 20 minutes in resting position, and considered IMP related.

Or

- If one or more subjects in the preceding dose group experience:
  - an increase in troponin I concentration which is considered clinically significant by the Investigator (with reference to the change from baseline value) and considered IMP related.

Or

- If the dose in a single subject is anticipated to exceed an $AUC_{0\text{-}tau}$ of ≥100 µg*h/mL. These limits are based on the exposure observed at the 20 mg/kg in cynomolgus monkeys (NOAEL: $AUC_{0\text{-}24}$ of 101 µg*h/mL in male and 111 µg*h/mL in females).

Or

- If the dose in a single subject is anticipated to exceed a C_{max} of ≥ 8.0 µg/mL. These limits are based on the exposure observed at the 20 mg/kg in cynomolgus monkeys (NOAEL: C_{max} of 8.03 µg/mL in males and 9.03 µg/mL in females).

Dose escalation between cohorts in Part A will be limited to a maximal 3-fold increase, and 2-fold increase between Part A and Part B, depending on safety and tolerability data and predicted exposure (both AUC and C_{max}).

If any of the above criteria are fulfilled, the cases will be discussed at the SRC who could proceed with partial or full unblinding as necessary to take its decision to either dose-escalate, proceed to enlargement of the cohort, or explore a lower dose.

If any of the stopping criteria occurred in a subject receiving DNDI-0690 the trial will put on halt and further dosing can be resumed only once an appropriate substantial amendment has been submitted and received regulatory and ethical approval from the relevant ethics committee and competent authority.

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

10.3.1 Part A

The starting dose for this multiple dose-escalation study was selected on the basis of the results of the SAD study in human healthy volunteers: at the highest administered dose (3600 mg), the $AUC_{0\text{-}inf}$ geometric mean was 47,800 (Geometric CV%: 26.7) ng.h/mL and the C_{max} geometric mean was 2560 (Geometric CV%: 20.7) ng/mL.

Predictions of steady state exposures have been performed simulating repeated doses at the actual dose levels administered in the SAD study in healthy male subjects in fasted condition. Actual single dose data at each dose level were used to predict its own steady state levels.

The performed non-parametric super positioning assumed linearity over time following repeated dosing. The following exposures were predicted for once a day dosing at steady state:

Table 4 Geometric Mean (CV%) Key Observed in the Single Ascending Dose Study and Predicted Pharmacokinetic Parameters of DNDI-0690 in Male Healthy Volunteers

DNDI-0690	Observed Parameters (CV%)				Predicted Parameters at Steady State after Once Daily Dosing (CV%)		
	Cmax (ng/mL)	AUC(0-12) (ng.h/mL)	AUC(0-24) (ng.h/mL)	AUC(0-inf) (ng.h/mL)	Cmax (ng/mL)	AUC(0-tau) (ng.h/mL)	Accumulation Ratio
10 mg	172 (26.9%)	1140 (46.3%)	1470 (61.4%)	1640 (77.9%)	182 (39.4%)	1570 (77.7%)	1.07 (13.9%)
30 mg	496 (29.1%)	3530 (40.1%)	4650 (50.1%)	5270 (62.0%)	534 (35.0%)	4980 (62.8%)	1.07 (11.5%)
150 mg	775 (25.6%)	5380 (49.4%)	7060 (66.8%)	7720 (78.4%)	827 (31.6%)	7240 (76.8%)	1.03 (8.09%)
400 mg	1120 (24.1%)	8500 (39.4%)	11800 (53.6%)	13900 (67.5%)	1240 (32.5%)	13100 (65.8%)	1.11 (10.6%)
1200 mg	1670 (25.2%)	13400 (35.2%)	19700 (48.9%)	25100 (63.9%)	1990 (37.2%)	23600 (64.2%)	1.20 (14.3%)
3600 mg	2600 (18.8%)	21200 (21.6%)	32800 (24.5%)	47100 (24.2%)	3380 (18.5%)	44900 (23.5%)	1.37 (6.63%)

Exposure after multiple dose of 400 mg OD are predicted to reach a mean (CV%) exposure of C_{max} 1240 (32.5%) ng/mL and an $AUC_{0-\tau}$ 13,100 (65.8%) ng.h/mL, which remains below the maximum exposure measured after single dose without safety concerns (see Table 4). The dose of 400 mg OD is thus considered a safe starting dose for multiple dosing.

Steady state is expected to be reached within 3 days maximum based on the observed geometric mean $t_{1/2}$ (ranging from 4.547 to 12.144 hours, depending on doses). Based on the minimally acceptable duration of VL treatment of 14 days as per the VL Combination therapy Target Product Profile, the duration of the present repeated dosing study was chosen to be 10 days. As steady state will likely be reached in 3 to 4 days, a duration of 10 days seems sufficient to describe the safety profile of DNDI-0690 in healthy volunteers.

Reversible and non-clinically relevant elevations of the creatinine levels were identified in healthy volunteers during the SAD. This pattern of slight increase in creatinine may be explained by an inhibition of creatinine secretion by cellular transporters. DNDI-0690 was assessed *in vitro* against a panel of drug transporters, showing 27.4 and 55.1 % inhibition of multidrug and toxin extrusion1 (MATE1; expressed in human embryonic kidney (HEK) cell line) at concentrations of 1 and 10 µM DNDI-0690, respectively. This effect could explain the

moderate increase in plasma creatinine which was observed in the SAD study. Of notice, MATE2 was unaffected in the *in vitro* studies.

Proper follow-up of renal function in the MAD, including the monitoring of plasma creatinine, BUN and biomarkers not affected by enzymatic transporters such as Cys C, will be ensured. A specific exploration of the renal function is proposed in part C of the MAD study with administration of iohexol, a non-ionic contrast agent used as marker freely filtrated by the glomerulus, and measure of its plasma clearance in order to establish the GFR in placebo and DNDI-0690 treated healthy subjects.

Dose escalation between cohorts will be limited to a 2-3-fold increase depending on safety and tolerability data and predicted exposure (both AUC and C_{max}).

The highest scheduled dose in Part A is set to 2400 mg/day. In addition, if safety stopping criteria are not met, a higher dose may be considered (up to a maximum of 3600 mg/day) for assessment of QT, but likely based on a shorter duration of treatment (see below Part B).

The animal data suggest that >95% efficacy can be achieved in all independent models when an $AUC_{cum,u}$ of 4.0 µg/mL*h was reached. Based on prediction of exposure in the MAD, a daily dose of 400 mg DNDI-0690 administered for 10 days may provide a highly efficacious treatment regimen. However, due to the variability in exposure between subjects seen in the SAD, the required exposure may not be reached for all subjects, and a dose up to 2400 mg may be required to achieve plasma levels at which efficacy is expected.

10.3.2 Part B

Safety pharmacology studies have shown that DNDI-0690 had no effects on CNS, respiratory function, and cardiovascular system. The absence of any effect on cardiovascular system was confirmed in vivo in the telemetry study in conscious cynomolgus monkeys, where no effects on cardiovascular parameters and body temperature were seen up to the single dose of 350 mg/kg.

There were no clinically significant findings in ECG results and no notable increases in QTcF interval in the SAD healthy volunteer study up to 3600 mg.

However, some heart changes as a consequence of nephrotoxicity were considered agonal in their nature and contributory to death together with renal changes in animal studies.

- Therefore, circulating levels of troponin I will continue to be monitored in the MAD study in healthy subjects at admission, at last day of treatment and in case of any ECG clinically significant abnormality during the course of treatment.
- In addition to safety ECGs, the effect of DNDI-0690 on Holter ECG parameters will be assessed in Part A and Part B.

Part B will only be implemented if the cardiac pharmacodynamic assessment (supra-therapeutic dose) is needed and if the SRC considers safe to proceed to a daily dosing above 2400 mg (with a maximum allowed dose of 3600 mg).

10.3.3 Part C

In line with the FDA's draft guidance for industry (U.S. Department of Health and Human Services 2017), additional evidence from clinical mechanistic studies is required in order to confirm whether or not the elevations in serum creatinine observed in the SAD study are due

to inhibition of the MATE-1 or other transporters. To address this, once a maximum tolerated dose of DNDi-0690 will be identified or at the highest dose tested, a cohort to calculate measured glomerular filtration rate (mGFR) will be conducted. mGFR data will be used for assessment of renal function. In the mGFR cohort, 9 subjects will receive multiple doses of DNDI-0690 or placebo once daily in a similar study design to the Part A cohorts, with the following specificities:

- Dose in Part C will already have been explored, no sentinel group will be implemented in this cohort.
- All subjects will receive 5 mL of iohexol solution (300 mg/mL iodine) intravenously on Day -1, Day 10, and optionally Day 24-28 that will be flushed with 10 mL of normal saline solution. On Day -1, iohexol will be administered at the same time as expected dosing of study drug on Day 10. On Day 10, iohexol will be administered immediately after dosing of study drug.
- Precaution for use as per iohexol summary of product characteristics (SmPC) will be respected[01].

10.4 Selection of Study Population

Up to fifty-four (54), 6 cohorts of 9 healthy male and healthy WONCBP subjects will be required to complete the study.

The study is to be conducted in healthy subjects; therefore, participants are not expected to derive any therapeutic benefit from taking part in the study. A healthy subject population with carefully considered inclusion / exclusion criteria will avoid the potential for interaction of DNDI-0690 with any underlying disease state or concomitant medication that it may be necessary for patients to take, while ensuring that subjects are fit and well enough for participation in the study.

The following eligibility criteria are designed to select subjects 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 subject.

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.

10.4.1 Inclusion Criteria

To be confirmed at Screening:

1. Healthy males or healthy women of non-childbearing potential (WONCBP) between 18 and 55 years of age inclusive at the time of signing informed consent.
2. Female subject of non-childbearing potential. WONCBP is defined as women who are postmenopausal or permanently sterilised (permanent sterilisation methods include hysterectomy, bilateral salpingectomy, and bilateral oophorectomy).

3. Female post-menopausal state is defined as no menses for 12 months without an alternative medical cause and confirmed by a serum FSH result of ≥40 IU/L at Screening.
4. Male subject (and subject's partner of childbearing potential) must use condom and also willing to use a highly effective method of contraception or 2 effective methods of contraception (see Section 10.5.2), if applicable (unless anatomically sterile or where abstaining from sexual intercourse is in line with the preferred and usual lifestyle of the subject) from first dose until 3 months after last dose of IMP.
5. Body mass index (BMI) of 18.0 to 30.1 kg/m2 as measured at Screening and body weight ≥60 kg (BMI = body weight (kg) / [height (m)]^2).
6. 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.
7. Subject with a negative urinary drugs of abuse (DOA) screen (including alcohol and 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).
8. Subject with negative human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg)) and hepatitis C virus antibody (HCV Ab) test results at Screening.
9. General good physical health determined by medical and surgical history, physical examination, 12-lead ECG, vital signs and clinical laboratory tests.
10. Normal blood pressure: Systolic blood pressure between ≥90 and ≤140 mmHg, Diastolic blood pressure ≤90 mmHg, measured after 10 min rest in supine position at Screening, admission and pre-dose.
11. A resting heart rate (HR) between ≥50 and ≤100 bpm measured after 10 min rest in supine position at Screening, admission and pre-dose.
12. ECG recording without clinically significant abnormality, including QTcF measure of ≤450 msec at Screening, admission and pre-dose.
13. No febrile seizures or infectious illness for at least 7 days prior to first administration of the IMP (Day 1).
14. Must agree to adhere to the contraception requirements and the life-style restrictions
15. Subject must be available to complete the study (including all follow up visits).
16. Subject must satisfy an Investigator about his/her fitness to participate in the study.
17. Subject must provide written informed consent to participate in the study.

To be re-confirmed on Day -2:

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

10.4.2 Exclusion Criteria

To be confirmed at Screening:

1. Subjects who have received any IMP in a clinical research study within 90 days prior to Day 1.
2. Subjects who are study site employees, or immediate family members of a study site or sponsor employee.
3. Subjects who have previously been enrolled in this study and/or have received DNDI-0690 previously.
4. History of any drug or alcohol abuse in the past 2 years.
5. Demonstrating excess in caffeine/xanthine consumption (more than 6 cups of coffee or equivalent a day).
6. Regular alcohol consumption in males >21 units per week and females >14 units per week (1 unit = ½ pint beer, or a 25 mL shot of 40% spirit, 1.5 to 2 Units = 125 mL glass of wine, depending on type). As confirmed by a positive urine alcohol test at Screening or admission.
7. Current smokers or those who have smoked within the last 12 months with a positive cotinine result at Screening and Admission.
8. Current users of cigarette replacements (*i.e.* e-cigarettes, nicotine patches or gums) and those who have used these products within the last 12 months.
9. Subjects who do not have suitable veins for multiple venepunctures/cannulation as assessed by the Investigator or delegate at Screening.
10. Clinically significant abnormal biochemistry, haematology, coagulation or urinalysis as judged by the Investigator or AST/ALT/total bilirubin/gamma glutamyl transpeptidase [GGT]/ALP/creatinine > 1.2 ULN. Subjects with Gilbert's syndrome are allowed.
11. Positive PCR COVID-19 test at admission.
12. Evidence of renal impairment at Screening or admission, as indicated by an estimated Glomerular Filtration Rate (GFR) < lower limit of normal (LLN) using the CKD-EPI equation.
13. History of clinically significant cardiovascular, renal, hepatic, neurological (especially seizures), immunological, psychiatric, myopathies, bleeding tendency, respiratory and particularly gastrointestinal (GI) disease, especially peptic ulceration and chronic gastritis, GI bleeding, ulcerative colitis, Crohn's Disease or Irritable Bowel Syndrome, as judged by the Investigator.
14. History of additional risk factors for Torsades des Pointe (*i.e.* heart failure, hypokalaemia, family history of long QT syndrome).
15. Rare hereditary problems of fructose intolerance, glucose-galactose malabsorption or sucrose-isomaltase insufficiency.
16. Any relevant GI complaints within 7 days of dosing.
17. Serious adverse reaction or clinically relevant hypersensitivity to any drug or the formulation excipients (Hypromellose [HPMC], sodium lauryl sulphate [SLS], sucrose, croscarmellose sodium and magnesium stearate).
18. Presence or history of clinically significant allergy requiring treatment (including asthma, urticaria, clinically significant allergic rash or other severe allergic diathesis), as judged by the Investigator. Hay fever is allowed unless it is active.

19. Donation or loss of greater than 500 mL of blood within the previous 3 months or more than 100 mL within 30 days prior to signature of informed consent.
20. Subjects who are taking, or have taken, any prescribed or over-the-counter drug (including anti-acid drugs) or vitamins/herbal remedies (*i.e.* St. John's Wort and others which are known to interfere with the CYP3A4 and P-gp metabolic pathways) or hormone replacement therapy (HRT) or any drug known to modify the MATE-1/OCT-2 renal transporters (such as, for example, cimetidine, ritonavir, trimethoprim, cisplatin) in the 28 days before IMP administration (or 5 half-lives of the taken drug, whichever is longer). Administration of up to 4 g of paracetamol per day within 7 days of IMP administration is allowed.
21. Surgery within 12 weeks prior to Screening, with the exception of appendectomy.
22. Any surgery (*i.e.* gastric bypass) or medical condition that may affect absorption of orally administered drugs.
23. Failure to satisfy the Investigator of fitness to participate for any other reason
24. Breastfeeding and lactating females.

Additional Exclusion Criteria for Part C

25. Hypersensitivity to iohexol or any of its excipients or to iodine, history of allergic reaction to iohexol, iodine or other contrast media, including cutaneous adverse reactions.
26. Manifest thyrotoxicosis.

To be re-confirmed at Day -2:

1. Development of any exclusion criteria since the Screening visit.
2. Use of prescription or non-prescription drugs, including vitamins, herbal and dietary supplements since Screening, unless in the opinion of the Investigator and Sponsor's Responsible Physician, the medication will not interfere with the study procedures or compromise subject safety.
3. Participation in a clinical study since the Screening visit.
4. Donation of 100 mL or more blood since the Screening visit.

10.5 Additional Advice and Restrictions for Study Population

10.5.1 Sperm Donation

Subjects must not donate sperm from the first dose and for at least 3 months after the last dose of IMP.

10.5.2 Contraception

There is a significant risk of drug exposure through the ejaculate (which also applies to vasectomised males) that might be harmful to the sexual partners (both male and female), including pregnant partners of male subjects.

Female subjects who are not of childbearing potential do not need to use any methods of contraception.

To prevent pregnancy, **male subjects** (and subject's partner of childbearing potential) must be using condom and also willing to use a highly effective method of contraception or 2 effective methods of contraception, if applicable (unless of non-childbearing potential or where abstaining from sexual intercourse was in-line with the preferred and usual lifestyle of the subject [periodic abstinence (*i.e.* calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception]) from the first dose until 3 months after the 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). For female subjects on the study, the vasectomised male partner should be the sole partner for that subject.

Effective methods of contraception include:

- Progestogen-only oral hormonal contraception (where inhibition of ovulation is not the primary mode of action),
- Male or female condom with or without spermicide,
- Cap, diaphragm or sponge with spermicide.

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

- Condom.

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

Male subjects must not have unprotected sexual intercourse with a female who is pregnant or breastfeeding during the study (up to 3 months post-last dose).

10.5.3 Diet and Fluid Restrictions

10.5.3.1 Mealtimes/Fasts

Once daily (OD) dosing regimen:

- Subjects will fast overnight for at least 10 hours (h) **before each morning 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.

Twice daily dosing (BID) regimen if needed:

- If a BID regimen is implemented, subjects will fast overnight for at least 10 hours (h) before each morning dose.
- The second, evening dose will be administered 10 hours following the morning dose. The second, evening dose shall be taken a minimum of 5 hours post mid-day meal, and one hour before the dinner.
- On Day 10, only a morning dose will be administered.
- Lunch will be served: approximately 4 h post-morning dose.
- Receive second, evening dose of the day: 10 h post-morningdose
- Dinner will be served: approximately 12 h post-morningdose.

On all non-dosing study days, whilst resident in the clinical unit, meals will be served at standard times. Subjects will choose meals from a standard menu while resident at the clinical unit.

Subjects are required to fast for at least 8 h or 10 h prior to laboratory sample collection for the following tests:

- For blood glucose tests (analysed in serum), require at least 8 h fasting period for fasted glucose test.

10.5.3.2 Fluid Intake

Part A and Part B: No fluids (apart from water taken with dose) are allowed from 1 h prior to dosing until 1 h afterwards.

OD dosing regimen: Water is then allowed ad libitum and at least 240 mL should be drinking every 4 h during waking hours (minimum of 1.5 L per day). Squash/cordial are allowed from 4 h post-dose and decaffeinated tea and coffee from 8 h post-dose.

BID dosing regimen: Water is then allowed ad libitum and at least 240 mL should be drunk every 4 h during waking hours until 1 h before the second, evening dose (minimum of 1.5 L per day). Squash/cordial are allowed from 4 h post morning dose and decaffeinated tea and coffee from 8 h post morning dose until 1 h before the second, evening dose.

Part C: 240 mL of water will be drinking after each urine PK timepoint on iohexol urine PK days (Day -1 and Day 10). Fluid intake on other study days will be similar to Part A and B.

10.5.3.3 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).

10.5.3.4 Caffeine

Food or drink containing caffeine or other xanthines, including coffee, tea, cola, energy drinks or chocolates will be avoided completely 3 days prior to dosing (24 hours prior to admission on Day -2) until discharge from the study.

10.5.3.5 Poppy and Sesame Seeds

Subjects will be advised that they must not eat food containing poppy or sesame seeds for 48 hours prior to Screening and for 48 hours prior to admission on Day -2 until discharge from the study, as consumption of poppy or sesame seeds can lead to a positive opiate result in the DOA test.

10.5.3.6 Grapefruit Juice and Other Restrictions

No food or drink containing grapefruit, cranberry, or Seville oranges (including marmalade and fruit juices), and/or food or drink, sweets, candies or other confectionary containing liquorice will be allowed from 7 days before the first dose of IMP until the final study visit.

10.5.4 Other Life-Style Restrictions

10.5.4.1 Strenuous Exercise

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

10.5.4.2 Blood Donation

Subjects will be advised that they should not donate blood or loss of greater than 500 mL of blood within the previous 3 months or more than 100 mL within 30 days prior to signature of informed consent.

10.5.5 Removal of Subjects from Therapy or Assessment

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

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

- If a subject experiences a serious or intolerable AE, that prevents them from continuing.
- If a subject 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 subject's health is compromised by remaining in the study or the subject is not sufficiently cooperative.
- If a subject is lost to follow-up.

The reasons for any subject withdrawal will be recorded on the study completion form of the case report form (CRF).

If a subject 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 6, Table 7 and Table 8). The data collected from withdrawn subjects will be included in the study report.

In the event of any abnormalities considered to be clinically significant, subjects 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.

Fifty-four (54) subjects are required to complete the study. Subjects who withdraw from the study before receiving any IMP will be replaced. Subjects who are withdrawn from the study due to significant drug-related AEs will not be replaced. Replacement of all other subjects withdrawn from the study after receiving IMP will be decided on a case-by-case basis by the PI (or deputy) and Sponsor.

10.6 Investigational Medicinal Product

10.6.1 Identity

The identity of each IMP is detailed in Table 5

Table 5 Identity of Investigational Medicinal Products

IMP Name	Strength	Presentation/Form	Route
DNDI-0690	100 or 200 mg of DNDI-0690	hard gelatine capsules	Oral
Matching Placebo	N/A	hard gelatine capsules	Oral

Non-IMP (NIMP): Iohexol solution (300mg/mL iodine).

10.6.2 Receipt and Storage

The IMP (DNDI-0690 and matching placebo) will be supplied by the Sponsor.

The investigational product will be supplied as hard gelatine capsules in HDPE bottles with induction seal closures. Each capsule contains either 100 mg or 200 mg of DNDI-0690. Each dose strength is presented as a Swedish orange, size 00 capsule. The investigational product also contains the following non-active ingredients: HPMC, SLS, sucrose, croscarmellose sodium and magnesium stearate.

A matching placebo formulation for all doses strengths is also provided. The placebo is made using the same non-active excipients as the investigational product (sucrose, croscarmellose sodium and magnesium stearate).

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 in the original container under quarantine in a segregated, study-specific area, at or below 30°C in a secure, temperature-controlled pharmacy. 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.

The NIMP, iohexol solution (300 mg/mL iodine) will be sourced by Simbec-Orion.

10.6.3 Assembly and Release

The IMP will be assembled into unit doses 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 guide to Good Manufacturing Practice (GMP)[06].

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

10.6.4 Administration

IMPs will be administered according to the following instruction:

- The IMPs will be administered fasted (after an overnight fast of at least 10 hours) with 240 mL water (more water will be provided if needed and the actual water taken with dose administration will be recorded in the eCRF). Subjects must remain upright for at least 4 h after dose, unless study procedures are performed.
- If a BID regimen is implemented, subjects will fast overnight for at least 10 hours (h) before each morning dose. The second, evening dose will be administered 10 hours following the morning dose. The second, evening dose shall be taken a minimum of 5 hours post mid-day meal, and one hour before the dinner. On Day 10, only a morning dose will be administered. (see section 10.5.3.1)
- A dose leader design will be implemented in **Part A and Part B** with 2 subjects being dosed on the first dosing day of EACH cohort. Of these 2, 1 will be on active drug and 1 on placebo. The remainder of the cohort will be dosed at least 48 hours later pending an acceptable safety profile in the dose-leader group and will contain 2 subjects on placebo and 5 subjects on active drug. This design allows maintenance of the "blind".

IMP administration will be documented in the CRF.

There will be at least 7 days between cohorts in Part A, and between Part A and Part B.

10.6.5 Return/Destruction

All used IMP containers and unused IMP will be held under quarantine pending return/destruction.

The Sponsor must provide approval for return/destruction of all remaining IMP within 8 weeks after study completion, provided final drug reconciliation activities are fully completed. 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.

10.6.6 Method of Assigning Subjects to Treatment Groups

Subjects 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 DMP). The randomisation code will include 2 dose-leaders (1 active:1 placebo) in each cohort in Part A and Part B.

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

10.6.7 Selection of Doses in the Study

The starting dose in Part A is once daily dose of 400 mg which was selected based the safety and PK data from the SAD study in human healthy volunteers. The rationale and justification of dose selection are detailed in Section 10.3.

10.6.8 Timing of Dose for Each Subject

OD doses will be administered at approximately 9.00 a.m. Subjects will be required to fast for at least 10 h overnight prior to each morning dose. The fast will be broken 4 h after dosing with a light lunch (see Section 10.5.3).

If a BID regimen is implemented, subjects will fast overnight for at least 10 hours (h) before each morning dose. The second, evening dose will be administered 10 hours after the morning dose. The second, evening dose shall be taken a minimum of 5 hours post mid-day meal, and one hour before the dinner. On Day 10, only a morning dose will be administered.

Two (2) dose leaders (1 active:1 placebo) will be dosed first in each cohort in Part A and Part B. The remainder of the cohort will be dosed a minimum of 48 hours later pending an acceptable safety profile in the dose-leader group.

10.6.9 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 and Sponsor personnel involved in the study will be blinded with regard to the IMP being administered. The pharmacist (or designee) responsible for the preparation of subject 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.

The bioanalytical scientist (at SGS), 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 subject number format, presented by dose level, in order to maintain the blind of study personnel.

Subject doses: Once the randomisation code has been authorised as per Simbec-Orion SOPs, each subject dose will be packaged and labelled for individual subjects by designated individuals from the IMP Management Department at Simbec-Orion on behalf of the Sponsor. The QP checks that the active drug and placebo are indistinguishable as part of the QP certification process.

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 subject. The envelopes will be stored in the restricted access pharmacy. A set of code break envelopes will also be provided to the person responsible for PV.

Emergency unblinding: Where the site requires emergency access to an individual subject code Simbec-Orion will break the blind via the code break envelopes stored in the pharmacy without prior consultation with the Sponsor. In such an event, the Sponsor will be notified as soon as possible via email.

Non-emergency unblinding: If an Investigator believes that knowledge of the IMPs received by a subject is essential for appropriate treatment of an AE, the code will be broken via the code break envelopes stored in the pharmacy. Where practical, the Investigator should ideally consult the Sponsor before breaking the code. In any event, Sponsor will be informed as soon as practical whenever the code has been broken for a subject.

If the blind needs to be broken for an individual subject, the date and reason will be recorded in the subject's CRF (the treatment allocation will not be captured in the CRF). Unless necessary, the Investigator will not reveal the unblinded treatment code to any other member of the clinical team involved in the study or to the Study Monitor. If the code is broken for any individual subject, the subject 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 GCP breach.

10.6.10 Prior and Concomitant Therapy

10.6.10.1 Prior Medication

Prescription or non-prescription drugs, including vitamins, herbal and dietary supplements should not be taken within 28 days (or 5 half-lives of the taken drug, whichever is longer) prior to the first dose of IMP, unless in the opinion of the Investigator and Sponsor's Responsible Physician the medication will not interfere with the study procedures or compromise subject safety. Prescription or non-prescription drugs, including vitamins, herbal and dietary supplements taken during the 28 days before the first dose of IMP, and the reason for taking them, will be noted in the subject's CRF.

Administration of up to 4 g of paracetamol per day within 7 days of IMP administration is allowed.

10.6.10.2 Concomitant Medication:

All drugs known to interfere with the renal transporters MATE1 and/or OCT2 shall be avoided (for example: cimetidine, ritonavir, trimethoprim, cisplatin) as they may create interferences in the assessment of the effect of DNDI-0690 on renal function.

Prescription or non-prescription drugs, including vitamins, herbal and dietary supplements should not be taken throughout the duration of the study, unless warranted by the treatment of AEs occurring after first IMP dose. In case of need for analgesia, paracetamol (which may be taken as an analgesic to a maximum of 2 g in 24 h) will be privileged, followed by ibuprofen (which may be taken as an analgesic to a maximum of 1.2 g in 24 h (400 mg 3 times a day)).

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 subject's CRF.

10.6.11Treatment Compliance

Each dose of IMP will be taken under supervision and a hand and mouth check conducted. The exact dosing time for each subject will be recorded on the subject's CRF.

10.7 Efficacy and Safety Variables

10.7.1 Flow Chart

A schedule of study assessments is provided in Table 6, Table 7 and Table 8.

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) and ± 5 minutes for samples collected > 30 minutes time-point.

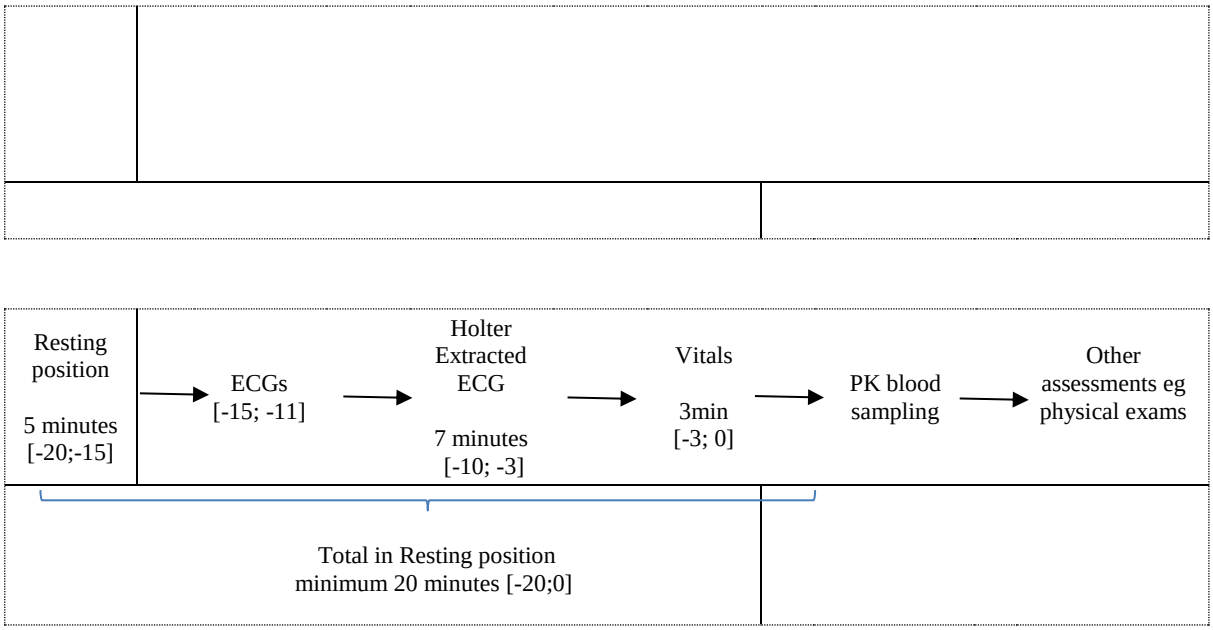
2. Holter extraction resting time. Start of resting time will be recorded.

3. Vital signs and 12-lead ECG (a window of ± 15 min in relation to the nominal time-point is allowed).

4. Start or finish of urine collection interval (a window of ± 10 min in relation to the nominal time-point).

5. All pre-dose assessments may be performed within the 1 h before dosing, except pre-dose urine samples can be collected outside this window.

As guidance, where Holter extracted ECGs coincide with other procedures *i.e.* vital signs and ECGs the order of procedures will be:



ECGs and Holter extractions should be taken prior to vital signs when both measurements are scheduled at the same time point. Holter extracted ECGs will be taken in the 10 min immediately prior to the nominal PK time point (window -10 to -3 minutes prior to PK timepoint) and no other procedures will be performed within this time window. Vital signs shall be taken prior to PK sampling. Other assessments, *i.e.* physical examinations etc, will be performed after PK sampling within the required time windows.

All safety assessments will be timed and performed relative to the start of dosing.

Table 6 Study Flow Chart: Part A

		Resident in the Clinical Unit														
	Screening	Admission			IMP Dosing Period										Discharge	Post Study Follow-up
Study Day	D-28 to D-3	D-2	D-1	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12 (last dosing +2 days)	D24-D28 (last dosing + 14-18 days)
General Assessments																
Informed consent	X															
Medical and surgical history	X															
Demographic Data	X															
Hepatitis/HIV serology	X															
PCR test COVID-19		X														
Inclusion/Exclusion criteria[1]	X	X		X	X	X	X	X	X	X	X	X	X			
Weight and height	X														X (Weight only)	
Vein assessment	X															
Urine drugs of abuse and alcohol/cotinine screen	X	X														
Randomisation				X												
IMP administration				X	X	X	X	X	X	X	X	X	X			

		Resident in the Clinical Unit														
	Screening	Admission		IMP Dosing Period											Discharge	Post Study Follow-up
Study Day	D-28 to D-3	D-2	D-1	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12 (last dosing +2 days)	D24-D28 (last dosing + 14-18 days)
Safety Assessments																
Physical examination[2]	X (Full)	X (Brief)					X (Brief)			X (Brief)					X (Brief)	X (Brief)
Laboratory safety tests (biochemistry, haematology, coagulation, urinalysis and urine microscopy)[3]	X	X			X		X			X			X		X	X
Troponin I[4]		X											X			
FSH (post-menopausal female subjects only)	X															
Holter monitoring (for extraction of ECGs)[5]			X										X			
12-lead ECG[6]	X	X		X			X			X			X		X	
Vital signs[7]	X	X		X			X			X			X		X	X
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serious adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Prior and concomitant medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

		Resident in the Clinical Unit													
	Screening	Admission		IMP Dosing Period										Discharge	Post Study Follow-up
Study Day	D-28 to D-3	D-2	D-1	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12 (last dosing +2 days)	D24-D28 (last dosing + 14-18 days)
Pharmacokinetic Assessments																
Plasma samples for DNDI-0690 and metabolite[8]				X	X	X	X	X	X	X	X	X	X	X	X	
DBS samples for DNDI-0690[9]				X									X	X	X	
Urine samples for DNDI-0690[10]				X			X			X			X			
Other assessments																
Urine samples for exploratory biomarkers[11]				X	X		X			X			X			
Full blood sampling for transcriptional profiling (mRNA) assessment (Part A Cohorts 2 & 3 only)[12]		X											X			

Study Flow Chart Footnotes:

1. Full inclusion/exclusion criteria will be checked at Screening. Eligibility will be re-confirmed on Day -2 and prior to each dose administration.
2. Physical examination: Full physical examination will be performed at Screening. A brief physical examination will be performed on Day -2, Day 4, Day 7, Day 12 and Post Study follow-up.
3. Laboratory safety tests will be performed at Screening, Day -2, pre-morning dose on Day 2/Day 4/Day 7/Day 10, Day 12 and Post Study follow-up. Glomerular Filtration Rate (GFR) will be estimated at Screening for eligibility purposes and at each time point, using the CKD-EPI equation. GFR based on Cys C results (GFRcc) will also be assessed.
4. Troponin I sample will be taken at Day -2 and Day 10 (pre--morning dose)
5. Holter monitoring will begin 60 minutes prior to the theoretical Day 1 morning dosing time on Day -1 and begin approximately 60 minutes prior to dosing on Day 10. Holter monitoring will end any time after Day 1 morning dosing and end any time after Day 11 (24 h post-last dose) PK sample collection. One set of triplicate ECG extractions to be taken at pre-morning dose (for baseline; T-30min to T-15 min) and at 0.5, 1, 2, 3, 3.5, 4, 6, 9, 12 and 24 h post-morning dose, time-matched on Day 10 timepoints for the Day -1 timepoints. Extractions are always done within the 10 minutes prior to PK sampling, except at pre-dose.

6. 12-lead ECGs will be taken at Screening, Day -2, Day 1/Day 4/Day 7/Day 10 (pre-morning dose and 4 h post-morning dose) and Day 12. Day 1, pre-morning dose ECG will be taken in triplicate (for baseline), all post-dose time points will be single ECG. An ECG can be repeated for any reason (technical in particular).
7. Vital signs (supine blood pressure, heart rate and Tympanic temperature) will be measured at Screening, Day -2, pre-morning dose on Day 1/Day 4/Day 7/Day 10, Day 12 and Post Study follow-up.
8. Plasma samples for DNDI-0690 and metabolite will be taken (4 mL K2EDTA tube) on Day 1 (pre-morning dose and 0.5, 1, 2, 3, 3.5, 4, 6, 9, 12 h post-morning dose), pre-morning dose on Day 2-Day 9 and Day 10 (pre-last dose and 0.5, 1, 2, 3, 3.5, 4, 6, 9, 12 h post-last dose), Day 11 (24 h post-last dose) and Day 12 (48 h post-last dose).
9. Dried blood spot (DBS) samples for DNDI-0690 will be taken (2 mL K2EDTA vacuum tube) on Day 1 (pre-morning dose, 0.5, 1 and 2 h post morning dose) and Day 10 (pre-last dose and 0.5, 1, 2, 3, 3.5, 4, 6, 9, 12 h post-last dose), Day 11 (24 h post-last dose) and Day 12 (48 h post-last dose).
10. Urine samples for DNDI-0690 will be collected on Day 1 (pre-morning dose and 0-24h post-morning dose) and on Day 4/Day 7/Day 10 (0-24 h post-morning doses).
11. Urine samples for exploratory biomarkers (Cys C, Kidney Injury Molecule-1 (KIM-1) and Neutrophil Gelatinase-Associated Lipocalin (NGAL)) will be collected at pre-morning dose on Day 1/Day 2/Day 4/Day 7/Day 10 (from morning spot collection).
12. Full blood sampling for transcriptional profiling (mRNA) assessment (Part A Cohorts 2 & 3 only) will be collected (2.5 mL vacutainer PAXgene® blood RNA tube) on Day -2 and Day 10 (pre-morning dose).

Table 7 Study Flow Chart: Part B

		Resident in the Clinical Unit									
	Screening	Admission		IMP Dosing Period						Discharge	Post Study Follow-up
Study Day	D-28 to D-3	D-2	D-1	D1	D2	D3	D4	D5	D6	D7 (last dosing +2 days)	D19-D23 (last dosing + 14-18 days)
General Assessments											
Informed consent	X										
Medical and surgical history	X										
Demographic data	X										
Hepatitis/HIV serology	X										
PCR test COVID-19		X									
Inclusion/Exclusion criteria[1]	X	X		X	X	X	X	X			
Weight and height	X									X (Weight only)	
Vein assessment	X										
Urine drugs of abuse and alcohol/cotinine screen	X	X									
Randomisation				X							
IMP administration				X	X	X	X	X			

		Resident in the Clinical Unit									
	Screening	Admission		IMP Dosing Period						Discharge	Post Study Follow-up
Study Day	D-28 to D-3	D-2	D-1	D1	D2	D3	D4	D5	D6	D7 (last dosing +2 days)	D19-D23 (last dosing + 14-18 days)
Safety Assessments											
Physical examination[2]	X (Full)	X (Brief)					X (Brief)			X (Brief)	X (Brief)
Laboratory safety tests (biochemistry, haematology, coagulation, urinalysis and urine microscopy)[3]	X	X			X		X	X		X	X
Troponin I[4]		X						X			
FSH (Post-menopausal female subjects only)	X										
Holter monitoring (for extraction of ECGs)[5]			X					X			
12-lead ECG[6]	X	X		X			X	X		X	
Vital signs[7]	X	X		X			X	X		X	X
Adverse events	X	X	X	X	X	X	X	X	X	X	X
Serious adverse events	X	X	X	X	X	X	X	X	X	X	X
Prior and concomitant medication	X	X	X	X	X	X	X	X	X	X	X

		Resident in the Clinical Unit									
	Screening	Admission		IMP Dosing Period						Discharge	Post Study Follow-up
Study Day	D-28 to D-3	D-2	D-1	D1	D2	D3	D4	D5	D6	D7 (last dosing +2 days)	D19-D23 (last dosing + 14-18 days)
Pharmacokinetic Assessments											
Plasma samples for DNDI-0690 and metabolite[8]				X	X	X	X	X	X	X	
DBS samples for DNDI-0690[9]				X				X	X	X	
Urine samples for DNDI-0690[10]				X				X			
Other assessments											
Urine samples for exploratory biomarkers[11]				X	X		X	X			

Study Flow Chart Footnotes:

1. Full inclusion/exclusion criteria will be checked at Screening. Eligibility will be re-confirmed on Day -2 and prior to each dose administration.
2. Physical examination: Full physical examination will be performed at Screening. A brief physical examination will be performed on Day -2, Day 4, Day 7 and Post Study follow-up.
3. Laboratory safety tests will be performed at Screening, Day -2, pre-dose on Day 2/Day 4/Day 5, Day 7 and Post Study follow-up. Glomerular Filtration Rate (GFR) will be estimated at Screening for eligibility purposes and at each time point, using the CKD-EPI equation. GFR based on Cys C results (GFRcc) will also be assessed.
4. Troponin I sample will be taken at Day -2 and Day 5 (Pre-dose).
5. Holter monitoring will begin 60 minutes prior to the theoretical Day 1 dosing time on Day -1 and begin approximately 60 minutes prior to dosing on Day 5. Holter recording will end any time after Day 1 dosing and end any time after Day 6 (24 h) post-last dose PK sample collection. One set of triplicate ECG extractions to be taken at pre-dose (for baseline; T-30min to T-15 min) and at 0.5, 1, 2, 3, 3.5, 4, 6, 9, 12 and 24 h post-dose, time-matched on Day 5 timepoints for the Day-1 timepoints. Extractions are always done within the 10 minutes prior to PK sampling, except at pre-dose.
6. 12-lead ECGs will be taken at Screening, Day -2, Day 1/Day4/Day 5 (pre-each dose and 4 h post-dose) and Day 7. Day 1, pre-dose ECG will be taken in triplicate (for baseline), all post-dose time points will be single ECG. An ECG can be repeated for any reason (technical in particular).
7. Vital signs (supine blood pressure, heart rate and Tympanic temperature) will be measured at Screening, Day -2, pre-dose on Day 1/Day 4/Day 5, Day 7 and Post Study follow-up.
8. Plasma samples for DNDI-0690 and metabolite will be taken (4 mL K2EDTA tube) on Day 1 (pre-dose and 0.5, 1, 2, 3, 3.5, 4, 6, 9, 12 h post-dose), pre-dose on Day 2/Day 3/Day 4 and Day 5 (pre-dose and 0.5, 1, 2, 3, 3.5, 4, 6, 9, 12 h post-dose), Day 6 (24h post-last dose) and Day 7 (48h post-last dose).
9. Dried blood spot (DBS) samples for DNDI-0690 will be taken (2 mL K2EDTA vacuum tube) on Day 1 (pre-dose, 0.5, 1 and 2 h post-dose) and Day 5 (pre-dose and 0.5, 1, 2, 3, 3.5, 4, 6, 9, 12 h post-dose), Day 6 (24 h post-last dose) and Day 7 (48 h post-last dose).
10. Urine samples for DNDI-0690 will be collected on Day 1 (pre-dose and 0-24h post-dose) and on Day 5 (0-24h post-dose).
11. Urine samples for exploratory biomarkers (Cys C, Kidney Injury Molecule-1 (KIM-1) and Neutrophil Gelatinase-Associated Lipocalin (NGAL)) will be collected at pre-dose on Day 1/Day 2/Day 4/Day 5 (from spot collection).

Table 8 Study Flow Chart: Part C

		Resident in the Clinical Unit														
	Screening	Admission	Iohexol Baseline	IMP Dosing Period											Discharge	Post Study Follow-up
Study Day	D-28 to D-3	D-2	D-1	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12 (last dosing +2 days)	D24-D28 last dosing + 14-18 days)
General Assessments																
Informed consent	X															
Medical and surgical history	X															
Demographic data	X															
Hepatitis/HIV serology	X															
PCR test COVID-19		X														
Inclusion/Exclusion criteria[1]	X	X		X	X	X	X	X	X	X	X	X	X			
Weight and height	X														X (Weight only)	
Vein assessment	X	X														
Urine drugs of abuse and alcohol/cotinine screen	X	X														
Randomisation				X												
Iohexol administration[2]			X										X			X (optional)
IMP administration				X	X	X	X	X	X	X	X	X	X			
Safety Assessments																
Physical examination[3]	X (Full)	X (Brief)	X (Brief)				X (Brief)			X (Brief)					X (Brief)	X (Brief)
Laboratory safety tests (biochemistry, haematology, coagulation, urinalysis and urine microscopy)[4]	X	X			X		X			X			X		X	X
Troponin I[5]		X		X									X			
FSH (post-menopausal female subjects only)	X															

		Resident in the Clinical Unit													
	Screening	Admission	Iohexol Baseline	IMP Dosing Period										Discharge	Post Study Follow-up
Study Day	D-28 to D-3	D-2	D-1	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12 (last dosing +2 days)	D24-D28 last dosing + 14-18 days)
12-lead ECG[6]	X	X	X	X			X			X			X		X	
Vital signs[7]	X	X	X	X			X			X			X		X	X
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serious adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Prior and concomitant medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pharmacokinetic Assessments																
Plasma samples for DNDI-0690 and metabolite[8]				X	X		X			X			X	X	X	
Plasma samples for iohexol[9]			X										X			X (optional)
Urine samples for iohexol[10]			X										X			X (optional)
Urine samples for exploratory biomarkers[11]				X	X		X			X			X			

Study Flow Chart Footnotes:

1. Full inclusion/exclusion criteria will be checked at Screening. Eligibility will be re-confirmed on Day -2 and prior to each dose administration.
2. Iohexol administration: All subjects will receive 5 mL of iohexol solution (300 mg/mL iodine) intravenously on Day -1, Day 10, and optionally on Day 24-28 (Post Study follow-up) that will be flushed with 10 mL of normal saline solution. On Day -1, iohexol solution will be administered at the same time as expected dosing of the study drug or placebo on Day 10. On Day 10, iohexol solution will be administered immediately after dosing of study drug or placebo.
3. Physical examination: Full physical examination will be performed at Screening. A brief physical examination will be performed on Day -2, Day -1, Day 4, Day 7, Day 12 and Post Study follow-up.
4. Laboratory safety tests will be performed at Screening, Day -2, pre-morning dose on Day 2/ Day 4/Day 7/Day 10, Day 12 and Post Study follow-up. Glomerular Filtration Rate (GFR) will be estimated at Screening for eligibility purposes and at each time point, using the CKD-EPI equation. GFR based on Cys C results (GFRcc) will also be assessed.
5. Troponin I samples will be taken on Day -2, Day 1 (pre-morning dose) and Day 10 (Pre-last dose).
6. 12-lead ECGs will be taken at Screening, Day -2, Day -1 (pre-iohexol dose), Day 1/Day 4/Day 7/Day 10 (pre-morning dose and 4 h post-morning dose) and Day 12. Day 1, pre-morning dose ECG will be taken in triplicate (for baseline), all post-dose time points will be single ECG. An ECG can be repeated for any reason (technical in particular).
7. Vital signs (supine blood pressure, heart rate and Tympanic temperature) will be measured at Screening, Day -2, Day -1 (pre-iohexol dose), pre-morning dose on Day 1/Day 4/Day 7/Day 10, Day 12 and Post Study follow-up.
8. Plasma samples for DNDI-0690 and metabolite will be taken (4 mL K2EDTA tube) at pre-morning dose on Day 1/Day 2/Day 4/Day 7 and Day 10 (pre-last dose and 0.5, 1, 2, 3, 4, 6, and 9 h post-last dose), Day 11 (24h post-last dose) and Day 12 (48h post-last dose).

9. Plasma samples for iohexol will be taken (5 mL lithium heparinized tube) at pre-iohexol dose, 1, 2, 3, 4 and 5 h post-iohexol dose on Day -1 and Day 10 and optional at Follow-up visit. Follow-up samples are needed only when results from previous timepoints of GFR calculation or urine microscopy showed abnormal results.
10. Urine samples for iohexol: Pre-iohexol dose, [0-1], [1-2], [2-3], [3-4] and [4-5] h post-iohexol dose on Day -1, Day 10 and optional at Follow-up visit. Follow-up samples are needed only when results from previous timepoints of GFR calculation or urine microscopy showed abnormal results.
11. Urine samples for exploratory biomarkers (Cys C, Kidney Injury Molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL)) will be collected at pre-morning dose on Day 1/Day 2/Day 4/Day 7/Day 10 (from morning spot collection).

10.7.2 Demographic and Background Assessments

The following demographic and background assessments will be performed during the study at the time-points specified in Table 6, Table 7 and Table 8.

10.7.2.1 Demographics

Demographic data: age, date of birth (only year of birth is recorded in the CRF), gender, race, height, weight and 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.

10.7.2.2 Medical and Surgical History

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

10.7.2.3 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.

10.7.2.4 PCR Test COVID-19

The POC Menarini vita PCR analyser will be used to determine the COVID-19 status using nasopharyngeal/oropharyngeal swab.

10.7.2.5 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 and cotinine 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.

10.7.2.6 Pregnancy Test, Menstrual and Obstetric History

Pregnancy test is not needed. Only WONCBP females will be included in this study.

Date and method of permanently sterilization will be recorded in the eCRF.

10.7.2.7 FSH

Serum FSH will be analysed from the same serum sample for biochemistry analyses at Screening for postmenopausal females only**.** **Serum** FSH analysis will be performed by Simbec-Orion Laboratory Services, or at an appropriate referral laboratory, using an appropriate analyser/method(s) of analysis.

10.7.2.8 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 -2.

10.7.3 Efficacy Assessments

Not applicable.

10.7.4 Safety Assessments

Safety assessments will be performed at the time-points specified in Table 6, Table 7 and Table 8.

10.7.4.1 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 required), 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 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 hospitalization or prolongation of existing hospitalization: *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 hospitalization 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, EMA and EC.

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.

10.7.4.2 Adverse Events

10.7.4.2.1 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.

10.7.4.2.2 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 in the following manner:

- 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 each 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.

10.7.4.2.3 Analysis of Events by the Sponsor

The Sponsor will also evaluate the seriousness of all events which are reported to him 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. Each SAE has to be classified by the Sponsor as expected or unexpected for the IMP.

10.7.4.2.4 Reporting Serious Adverse Events

The Investigator is required to notify the study sponsor and pharmacovigilance provider if appropriate within 24 h of becoming aware of the occurrence of an SAE. A copy of the written report of the event should promptly be sent to the study Sponsor for information purposes, 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);
- immediately inform (within 24 h of awareness of SAE by the Investigator) by telephone, the Sponsor Medical responsible, and the Sponsor Clinical Project Manager, and confirmed by an email to **pharmacovigilance@dndi.org (copy SAE_DNDI0690@dndi.org)**
- complete the SAE form and send it by email **to pharmacovigilance@dndi.org (copy SAE_DNDI0690@dndi.org),** immediately after of being informed of this event, without waiting for the results of the clinical outcome or of 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;
- 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.

10.7.4.2.5 Reporting of SUSARs

The Sponsor is responsible for all declarations to Health Authorities (EMA/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 (represented by the pharmacovigilance service provider [United BioSource Corporation] by delegation) is responsible for reporting SUSARs to the EC.

10.7.4.2.6 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 and EMA as soon as possible, but no later than 7 calendar days after they 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 pharmacovigilance provider.

The Sponsor (represented by the pharmacovigilance service provider [United BioSource Corporation] by delegation) is required to notify the EC of any fatal or life-threatening SUSAR as soon as possible, but no later than 7 calendar days after they 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 pharmacovigilance provider.

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

10.7.4.2.7 Reporting of Urgent Safety Issues

Simbec-Orion is required to inform the appropriate competent authorities and the EC within 3 calendar days of the urgent safety issue.

10.7.4.2.8 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 partners, 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.

10.7.4.2.9 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.

10.7.4.2.10 Monitoring of Subjects with Adverse Events

In the event of any abnormalities considered to be clinically significant by the investigating physician, 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.

10.7.4.3 Pregnancy

The following procedures should be followed if the partner of a subject becomes pregnant. Only WONCBP are to be enrolled in this study, however, in the unlikely event that a female subject or the partner of a male subject becomes pregnant during the study, these procedures should also be followed.

- Subjects will be instructed that if they/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/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 the subject/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.

10.7.4.4 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 laboratory services plan (LSP).

10.7.4.4.1 Biochemistry Tests

- Alanine Aminotransferase (ALT)
- Albumin
- Alkaline Phosphatase (ALP)
- Aspartate Aminotransferase (AST)
- Bicarbonate
- Total Bilirubin
- Bilirubin (Direct) (only if Total is elevated)
- Calcium (Ca)
- Chloride (Cl)
- Creatine Kinase (CK)
- Creatinine (enzymatic and normalized)*
- Follicle Stimulating Hormone (FSH; post-menopausal female subjects only)
- Gamma Glutamyl Transferase (GGT)
- Glucose (Fasting)
- Potassium
- Phosphate (Inorganic)
- Protein (Total)
- Sodium (Na)
- Urea
- Lactate dehydrogenase (LDH)
- Blood Urea Nitrogen (BUN)
- Uric acid
- GLDH
- Cholesterol
- Triglycerides
- Cystatin C (Cys C in serum)*

*Glomerular Filtration Rate (GFR) will be estimated at Screening for eligibility purposes and at each time point, using the CKD-EPI equation. GFR based on Cys C results (GFRcc) will also be assessed.

- CKD-EPI equation[09]:
    - **eGFR** = 141 * min (Cr/κ,1)α X (max(Cr/κ,1)$^{-1.209}$) X (0.993^{Age}) X 1.018 [if female] X 1.159 [if black]

      Where:

      Cr- is serum creatinine in mg/dL;

      k is 0.7 for women, 0.9 for men;

α is -0.329 for women and –0.411 for men.

min indicates the minimum of Cr/κ or 1

max indicates the maximum of Cr/κ or 1

- GFRcc equation[010]:
  - $eGFR_{CystC} = 130 \times \text{cystatin C}^{-1.069} \times \text{age-}\ 0.117 - 7$

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.

10.7.4.4.2 Troponin I (serum)

Troponin I will be run on the biochemistry sample if possible.

A separate tube (Serum Storage Tube, 3.5mL) for troponin I is needed at timepoints when biochemistry is not required.

10.7.4.4.3 Haematology Tests

- Haemoglobin
- Haematocrit
- Mean Corpuscular Volume (MCV)
- Mean Corpuscular Haemoglobin (MCH)
- Mean Corpuscular Haemoglobin Concentration (MCHC)
- Platelet Count, Red Blood Cell Count (RBC)
- Red Blood Cell Distribution Width (RDW), 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.

10.7.4.4.4 Coagulation Tests

- Activated partial thromboplastin time (aPTT)
- prothrombin time (PT)
- international normalised ratio (INR)
- Thrombin time

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.

10.7.4.4.5 Urinalysis Tests:

- Bilirubin
- Blood
- Glucose
- Ketones
- Leukocytes
- Nitrites
- pH
- Protein
- Specific gravity
- Urobilinogen
- Microalbuminuria
- Creatininuria
- Urine Microscopy (to be performed on all urine samples)

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.

The following test parameters will be reported for urine microscopy: bacteria, casts (non-pathogenic), casts (pathogenic), crystals, epithelial cells, RBCs and WBCs. 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.

10.7.4.5 Vital signs

Supine systolic/diastolic blood pressure, pulse rate, tympanic temperature.

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

10.7.4.6 Physical Examination

A physical examination will be performed by an Investigator. The 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.

10.7.4.7 12-lead ECG

HR, PR interval, QRS width, QT interval and QT interval corrected using Bazett's formula (QTcB) and 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, and date of birth (Year only). An Investigator will provide an interpretation of each tracing **within 2 hours (Pre-dose ECGs need to be reviewed before dosing).** Clinically significant abnormalities will be recorded on the AE page. Normal ranges for 12-lead ECG parameters are presented in Appendix 1.

- All safety ECG data (from baseline to end of study visit) will be sent to the central ECG reading facility (Banook) for review by the cardiologist. A cohort report on ECG findings will be issued for review at the SRC dose-escalation meetings. Measurements will be sent back to Simbec-Orion at the end of the study to be included in the database.
- All safety ECG shall be done after the volunteer being in resting position for a minimum of 5 minutes.

10.7.4.8 ECG Holter

Holter monitoring will be performed in Part A and Part B.

One set of triplicate ECG extractions to be taken at pre-dose (for baseline; T-30min). Holter extraction time points on each day of Holter monitoring will be at pre-dose and 0.5, 1, 2, 3, 3.5, 4, 6, 9, 12 and 24 h post-dose, time-matched on Day10 timepoints for the Day-1 timepoints. Extractions are always done within the 10 minutes prior to PK sampling.

Continuous Holter monitoring will commence at least 1 h prior to dosing until 24 h post-dose, i.e, after the blood sample for PK has been withdrawn at 24 h post-last dose. Subjects will be required to be supine for 20 min *(10 min rest period, 10 min extraction)* prior to the nominal time point where an ECG extraction is scheduled. The extraction will be taken in the 10 min immediately prior to the nominal time point and no other procedures will be performed within this time window. All ECG extractions will occur in triplicate. Values from triplicates (RR, PR, QRS, and QT) will be averaged in order to obtain one single value per time point.

- The first extraction will be scheduled in a window of -30 to -15 minutes prior to dosing, volunteers need to be in supine position for at least 10 minutes prior to defined extraction window.

The recordings will be archived for a maximum of 25 years and analyzed only in case the clinical development of DNDI-0690 progresses to a Phase II trial. The Holter extracted ECGs will be analysed together with the Holter extracted ECGs collected in the SAD trial and reported in a separate PK/PD report.

10.7.4.9 Concomitant Medication

All prior and concomitant medications taken during the study will be recorded in the subject's CRF.

10.7.5 Appropriateness of Measurements

All measurements performed in the study are standard measurements.

The total volume of blood to be collected from each subject during the study are presented in Table 9 below which is considered acceptable.

Table 9 Summary of Blood Volume

Procedure	Visit	Number of Samples	Blood Volume per Sample (mL)	No. Treatment Days	Blood Volume (mL)
Biochemistry[1]	Screening	1	4.5	N/A	4.5
	Days -2 /2/4/7/10/12 (Part A)	6	4.5	N/A	27
	Days -2/2/4/5/7 (Part B)	5	4.5	N/A	22.5
	Days -2/2/4/7/10/12 (Part C)	6	4.5	N/A	27
	Post study follow-up	1	4.5	N/A	4.5
Haematology	Screening	1	3	N/A	3
	Days -2 /2/4/7/10/12 (Part A)	6	3	N/A	18
	Days -2/2/4/5/7 (Part B)	5	3	N/A	15
	Days -2/2/4/7/10/12 (Part C)	6	3	N/A	18
	Post study follow-up	1	3	N/A	3
Coagulation	Screening	1	2.7	N/A	2.7
	Days -2 /2/4/7/10/12 (Part A)	6	2.7	N/A	16.2
	Days -2/2/4/5/7 (Part B)	5	2.7	N/A	13.5
	Days -2/2/4/7/10/12 (Part C)	6	2.7	N/A	16.2
	Post study follow-up	1	2.7	N/A	2.7
Plasma samples for DNDI-0690 and metabolite	Day 1: 10 samples, days 2-9 and Day 10: 12 samples (Part A)	30	4	N/A	120
	Day 1: 10 samples, days 2/4 and Day 5: 12 samples (Part B)	24	4	N/A	96
	Day 1: 1 sample, days 2/4/7 and Day 10: 8 samples (Part C)	12	4	N/A	48
DBS samples for DNDI-0690	Day 1: 4 samples and Day 10: 12 samples (Part A)	16	2	N/A	32
	Day 1: 4 samples and Day 5: 12 samples (Part B)	16	2	N/A	32
Full blood sampling for transcriptional profiling (mRNA) assessment	Day -2 and Day 10 (Part A Cohorts 2&3)	2	2.5	N/A	5

(Part A cohorts 2&3)					
Plasma samples for iohexol	Day -1 and Day 10: 6 samples (Part C) – FU (optional 6 samples)	18	4	N/A	72
			Total blood[2/3]	Part A (Cohorts 1 & 4)	233.6
				Part A (Cohorts 2 & 3)	238.6
				Part B	199.4
				Part C	201.6

[1] From the biochemistry blood sample collected at the Screening visit and virology screen will be analysed from the same serum sample. Troponin I will be analysed using the biochemistry samples.
[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.
[3] The exact volumes of each sample may change but the total volume of blood drawn for any subject will not exceed 450 mL.

10.7.6 Primary Efficacy Variable(s)

N/A

10.7.7 Drug Concentration Measurements

Plasma and urine samples for drug concentration measurements will be analysed at SGS, using a validated liquid chromatography tandem mass spectrometry (LC MS/MS) method, according to applicable local SOPs.

Metabolite identification is exploratory.

10.7.7.1 Pharmacokinetic Blood Sampling for DNDI-0690 and Metabolite

The first 0.5 mL of blood withdrawn via cannula will be discarded.

Blood sample (4 mL venous blood) for the determination of plasma DNDI-0690 and metabolite levels will be collected into K2EDTA tube(s) per time-point as specified in Table 6, Table 7 and Table 8:

- Immediately after sample collection, each sample collection tube will be identified with a barcoded label bearing details of the study number, subject number, sampling time-point, sample type (*i.e.* sample matrix, *e.g.* blood, etc), and a unique 9-digit sample identification number.
- The collected sample will then be processed within 30 mins of collection by centrifugation at 1500 g for 10 min at 4°C.
- Transfer plasma into 2 appropriately labelled 3.5mL polypropylene tubes. each labelled similarly to the original blood sample [bearing the same details of the study number, subject number, sampling time-point, and unique 9-digit sample identification number, except for the sample type (*i.e.* sample matrix, *e.g.* serum, plasma etc)],
    - For each samples, 2 tubes should contain at least 500µL of plasma.
- Freeze samples as soon as possible after processing (samples should be frozen within 4 hours of collection)

- Store the samples at - 20°C or below (no lower than -35°C) until shipment.

The date and time at which each PK sample are collected, received in the separating room, and subsequently placed in the appropriate freezer(s) will be recorded in the study documentation.

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.

10.7.7.2 Pharmacokinetic Urine Collection for DNDI-0690

Urine samples will be collected for the determination of DNDI-0690 level in urine at the time-points specified in Table 6 and Table 7.

Each urine collection container will be identified with a barcoded label bearing details of the study number, subject number, sampling time-point, sample type (*i.e.* sample matrix, *e.g.* blood, etc), and a unique 9-digit identification number.

- Only a single urine void is required pre-dose, each subject will collect one sample pre-dose (ideally the first void of the day prior to dosing). Subjects will be encouraged to empty their bladder immediately prior to dosing; however, this void will not be required to be collected if a sample has already been produced (first void of the day).
- The urine collection containers will be kept chilled (at approximately 4ºC) during the collection intervals.
- The empty container should be weighed prior to collection. At the end of the collection timepoint, the bulk sample should be weighed (g) and the volume calculated (v). Once weighed, thoroughly mix the urine and immediately, using a Gilson pipette, transfer **exactly** 1 mL of urine into 2 appropriately labelled polypropylene 3.5ml tubes each containing 1 mL of an aqueous solution of BSA/NaCl 50 g/L and NaCl 9 g/L. Volumes must be accurate.
- 2 x 2 mL aliquots (containing 1 mL urine and 1 mL BSA/NaCL solution) will be transferred immediately to the -20°C freezer until shipment to SGS for analysis.

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

10.7.8 DBS Samples for DNDI-0690

2 mL whole blood will be collected from the forearm vein using the K2EDTA vacuum 2 mL tubes at the time-points in Table 6 and Table 7.

- Immediately after sampling, homogenise gently by inverting the tube at least 15 times, without shaking it, to mix the blood with the anticoagulant.
- The whole blood samples will be processed within 30 minutes of sample collection.
    - Ensure sampling tubes are clearly and correctly labelled with a barcoded label bearing details of the study number, subject number, sampling time-point, sample type (i.e. sample matrix, *e.g.* DBS, etc), and a unique 9-digit identification number.

- Never allow the tube to rest. Always maintain a gentle agitation until depositing blood on the DBS card.

The DBS sample processing method will be detailed in the SHM. The DBS samples will be shipped to SGS for analysis.

The DBS samples are exploratory and will be analysed when the analytical method is fully validated.

10.7.9 Full Blood Sampling for Transcriptional Profiling (mRNA) assessment

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 6.

- 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.

10.7.10 Plasma Samples for Iohexol

Blood sample (4 mL) for the determination of plasma iohexol levels will be collected into lithium heparinized tube(s) per time-point as specified in Table 8.

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

10.7.11 Urine Samples for Iohexol

Urine samples will be collected for the determination of iohexol in urine at the time-points specified in Table 8.

Each urine collection container will be identified with a barcoded label bearing details of the study number, subject number, sampling time-point, sample type (*i.e.* sample matrix, *e.g.* blood, etc.), and a unique 9-digit identification number.

- Only a single urine void is required pre-dose, each subject will collect one sample pre-dose (ideally the first void of the day prior to dosing). Subjects will be encouraged to empty their bladder immediately prior to dosing; however, this void will not be required to be collected if a sample has already been produced (first void of the day).
- The urine collection containers will be kept chilled (at approximately 4ºC) during the collection intervals.
- At the end of the collection timepoint, the bulk sample should be weighed (g) and the volume calculated (v). Once weighed, thoroughly mix the urine and immediately, using a Gilson pipette, transfer 10 mL of urine into 2 appropriately labelled polypropylene tubes.
- 2 x 10 mL aliquots will be transferred immediately to the -20°C freezer until shipment for analysis.

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

10.7.12 Urine Samples for Exploratory Biomarkers

Urine samples (from PK urine samples or spot collection) will be collected for the following exploratory markers at the time-points specified in Table 6, Table 7 and Table 8.

- Kidney Injury Molecule-1 (KIM-1)
- Neutrophil Gelatinase-Associated Lipocalin (NGAL)
- Cystatin C (Cys C)

Urine samples for the determination of exploratory biomarkers will be collected from a morning spot urine sample or at the time of urine collection for safety sampling.

2 x 20 mL samples of urine will be aliquoted from the bulk urine in to 2 appropriately labelled 25 mL polypropylene tubes immediately after the urine has been mixed. These aliquots must be taken after the bulk sample has been weighed and volume calculated. Samples should be stored at -80°C until shipment

After all aliquots for bioanalysis, urine iohexol analysis and exploratory biomarkers for each collection timepoint have been removed, the remaining bulk sample will be discarded.

Analysis of urine exploratory biomarkers will be optional and a decision to analyse those samples will be made by DNDi at the end of the study.

The biomarkers will be analysed by Simbec-Orion Laboratories if required. For biomarker tests the urine creatinine level is also required in order to normalise the data to account for differences in urine volume of a sample.

- Urine NGAL levels will be measured on the automated Roche c501 immunoassay platform using commercially available Bioporto NGAL immunoassay kit reagents.
- Urine Kim-1 level will be measured using a commercially available R&D System ELISA kit.
- Urine Cys C level will be measured using a commercially available R&D System ELISA kit.

The exploratory biomarker samples will be batched prior to analysis.

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

10.7.13 Other Assessments

N/A

10.8 Data Quality Assurance

At the time the study is initiated, a representative of the Sponsor will thoroughly review the final protocol and CRFs 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 subjects' records (including the volunteer (subject) master files, laboratory and 12-lead ECG print-outs), the accuracy of entries into the CRFs, 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), Sponsor representatives, the REC, and the MHRA direct access to relevant clinical records to confirm their consistency with the CRF entries. No information in these records about the identity of the subjects will leave Simbec-Orion. The Sponsor will maintain the confidentiality of all subject records, in line with Section 6.10 of the ICH GCP E6 (R2) guidelines[02].

Study data will be fully documented in the CRFs 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 10.

Table 10 Summary of Source Documentation Location

Data	Source Document			
	Volunteer Master File	**eCRF[1]***	**Paper Workbook**	**Other**
Evidence of healthy subject status/primary disease condition for entry into clinical study	X			
Demographic data		X		
Medical and surgical history and age	X			
Inclusion and exclusion criteria/subject eligibility[2]		X	X	
Informed consents[3]		X		X
Subject participation in the clinical study		X		
Screening number				X (Screening log)
Subject number				X (Enrolment log)
AEs		X		
SAEs				X (SAE form)
Pregnancies				X (Pregnancy notification and outcome form)
Previous and on-going therapy	X	X		
Concomitant medication		X		
Meals start and stop time				X (Meal Logbook)
Daily water consumption volume				X (Water Intake Logbook)
Results of study examinations (*e.g.* physical examination, 12-lead ECGs, vital signs and laboratory safety tests)[4]		X		X
Study visit dates		X		
Administration of IMP		X		
Blood PK sample collection times		X		
Urine PK sample collection times (including start and stop times of collection intervals and volumes)				X (logbook)
Blood safety sample collection times		X		
Urine safety sample collection times (including start and stop times of collection intervals and volumes)				X (logbook)
ECG Holter				X (Holter extractions)
Physical examination[5]		X		

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 by Simbec-Orion clinical staff.
2. Subject eligibility for this study will be captured in an inclusion/exclusion table found in the paper Source Workbook.
3. 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. Details of consent *e.g.* date, version, Physician who consented, will be entered on eCRF.
4. The 12-lead ECG trace and laboratory safety test print-out including medical review will be stored with the Source Data Files.
5. The date and time of each physical examination will be recorded in the eCRF. Abnormal physical examination results will be recorded on medical history page at Screening or on AE page post dosing.

CRF = case report form, ECG = electrocardiogram, PK = pharmacokinetic

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 measures from the clinical study that, in the opinion of an Investigator, should be noted.
- Any concomitant medications used to treat the subject during the study that, in the opinion of an Investigator, should be noted.

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

Designated Investigator site staff will enter the data required by the protocol directly into the electronic CRFs (e-CRFs) 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 e-CRF 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 SOPGRP-QA 002.

Independent clinical quality assurance 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.

10.9 Statistical Methods and Determination of Sample Size

10.9.1 Statistical Analysis Plan

A statistical analysis plan (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.

10.9.2 Study Variables/Endpoints

10.9.2.1 Primary Endpoints

Safety parameters (changes in vital signs, ECG, safety laboratory parameters with special focus on renal function parameters, *i.e.* serum creatinine, blood urea nitrogen (BUN), and Cys C), and frequency and severity of observed TEAEs.

10.9.2.2 Secondary Endpoints

Measurement of the following PK parameters:

- Day 1:

- Main: C_{max}, C_{max}/D, AUC_{0-24}, AUC_{0-24}/D
- Exploratory: $AUC_{0-24,norm}$, $C_{max,norm}$, T_{max}, MRT_{last}

- Day 10:
  - Main: AUC_{0-inf}, AUC_{0-inf}/D, AUC_{0-24}, AUC_{0-24}/D, $C_{max\ ss}$, $C_{max\ ss}$/D, $C_{min\ SS}$
- Exploratory: $AUC_{0-inf,norm}$, $AUC_{0-24,norm}$, AUC_{last}, AUC_{last}/D, $AUC_{last,norm}$, $C_{max\ ss,norm}$, T_{max}, $t_{½}$, Cl/F, lambda-z, MRT_{last}, Vz/F, CL_{ss}/F and points terminal for DNDI-0690
- Optional: if BID dosing, AUC_{0-10}, AUC_{0-10}/D, $AUC_{0-10,norm}$ will be added on Day 1 and Day10
- AUC_{t-inf}, $\%AUC_{extra}$, points terminal
- Accumulation ratios RA(C_{max}) and RA(AUC_{0-tau}) will be calculated
- C_{trough} will be derived from the concentration data (Days 2 – 10, Part A, days 2/4/5 for Part B and days 2/4/7/10 for Part C)
- Measurement of the urine PK parameters Ae, Ae% and CL_R for DNDI-0690
- Glomerular filtration rate measurement (mGFR) following evaluation of plasma clearance of iohexol before and under exposure to DNDI-0690

10.9.2.3 Exploratory Endpoints

- Changes in clinical early renal toxicity markers in urine: Cystatin C (Cys C), Kidney Injury Molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL).
- Analysis of Holter extracted ECGs for the following parameters: RR, heart rate (HR), PR, QRS, QT, corrected QT interval by Fridericia's formula (QTcF), corrected QT interval by Bazett's formula (QTcB), ΔHR, ΔRR, ΔPR, ΔQRS, ΔQT, ΔQTcF and ΔQTcB.
- Measurement of drug concentration in dry blood spots for comparison with matching plasma samples.
- Variation of mRNA expression in blood sample before and after exposure to the drug (Transcriptional Profiling).
- Exploration of metabolism after multiple dose exposure.

10.9.2.4 Analysis Sets

Safety Set: All randomised subjects who receive at least 1 dose of IMP will be included in the safety analysis.

PK Set: Subjects will be assigned to the PK set on a per treatment basis. Randomised subjects 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 an occurrence of vomiting (that occurs at or before 2 times median T_{max} within the appropriate cohort or treatment) or diarrhoea which renders the concentration profile unreliable,

- Do not have an occurrence of vomiting (during a period of time less than or equal to the dosing interval) or diarrhoea which renders the concentration profile unreliable,
- Do not use a concomitant medication which renders the concentration profile unreliable,
- Do not have a pre-dose concentration that is greater than 5% of the corresponding C_{max},
- Have at least one pharmacokinetic 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).

10.9.2.5 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 DMP/SAP).

10.9.2.5.1 Demographic and Background Data

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

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

Demographics: Demographic data will be listed. Descriptive statistics (number of subjects in the analysis set (N), number of subjects 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.

10.9.2.5.2 Efficacy Data

N/A

10.9.2.5.3 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 DMP). The MedDRA dictionary will not be updated during the course of the study.

All AEs and SAEs, including those which occurred prior to the first dose of IMP, will be listed. Only 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.

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

The number of TEAEs and the number and % of subjects reporting at least 1 TEAE will be tabulated by system organ class (SOC) and preferred term. A subject 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 subjects reporting at least 1 TEAE will be tabulated by preferred term and sorted by descending frequency on the total number of subjects with that AE. A subject 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 subjects reporting TEAEs will be tabulated by maximum severity and strongest relationship to IMP. For the summary of TEAEs by severity, if a subject 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 subject 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 -2) 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, 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 QTcB and QTcF values will be summarised according to the below categories:

For absolute values

- QTcB/ QTcF ≤ 450 msec.
- QTcB/ QTcF > 450 msec and QTcB/ QTcF ≤ 480 msec.
- QTcB/ QTcF > 480 msec and QTcB /QTcF ≤ 500 msec.
- QTcB/ QTcF > 500 msec.

For change from baseline

- QTcB/ QTcF ≤ 30 msec.
- QTcB/ QTcF > 30 msec and QTcB/ QTcF ≤ 60 msec.
- QTcB/ QTcF > 60 msec.

10.9.2.5.4 PK Data

Concentration-Time Data: Individual plasma DNDI-0690 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.

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.

In addition, an investigative plasma analysis will be performed to further evaluate a potential metabolite M5 (no chemical structure could be assigned but likely formed via di-oxidation and nitro-reduction) which was detected in plasma of preclinical species and in exploratory investigations in humans. However, absolute circulating amounts of M5 remain unknown since no reference standard of M5 is available due to its instability following chemical synthesis.

Derived PK Data: The following PK parameters will be derived from plasma DNDI-0690 concentration-time data following administration of IMP 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 zero.

- **Day 1 (Part A and Part B):**
    - C_{max}: Maximum concentration
    - C_{max}/D: Maximum concentration/Dose
    - AUC_{0-24}: Area under the concentration-time curve (AUC) from the time of dosing to 24 hours pose
    - AUC_{0-24}/D: AUC_{0-24}/Dose
    - $AUC_{0-24,\ norm}$: Area under the concentration versus time curve from zero to 24 hours divided by dose per kilogram body weight
    - $C_{max,\ norm}$: Maximum concentration divided by dose per kilogram body weight
    - T_{max}: The time to maximum observed concentration
    - MRT_{last}: Mean Residence Time from the time of dosing to the time of the last measurable concentration
    - Ae_{0-24}: Amount excreted in urine over each collection interval (0 to 24 hours post-dose).
    - Ae%: % of dose excreted in urine over each collection interval (0 to 24 h post-dose).
    - CL_R: Renal clearance
- **Day 4 (Part A and Part B) and Day 7 (Part A)**

- Ae_{0-24}: Amount excreted in urine over each collection interval (0 to 24 hours post-dose on Day 4 and Day 7).
- Ae%: % of dose excreted in urine over each collection interval (0 to 24 h post-dose on Day 4 and Day 7).

- **Day 10 (Part A and Part C) and Day 5 (Part B):**
  - AUC_{0-inf}: AUC extrapolated to infinity
  - AUC_{0-inf}/D: AUC extrapolated to infinity/Dose
  - AUC_{0-24}: Area under the concentration-time curve (AUC) from the time of dosing to 24 hours pose
  - AUC_{0-24}/D: AUC_{0-24}/Dose
  - $C_{max,ss}$: Maximum concentration at steady state
  - $C_{max,ss}$/D: Maximum concentration at steady state/Dose
  - $C_{min,SS}$: Minimum concentration at steady state
  - $C_{av,SS}$: Average concentration at steady state
  - Degree of fluctuation: $(C_{max}-C_{min})/C_{av,SS}$
  - Swing: $(C_{max,SS}-C_{min,SS})/C_{min,SS}$
  - $AUC_{0-inf,norm}$: AUC extrapolated to infinity divided by dose per kilogram body weight
  - $AUC_{0-24,\ norm}$: Area under the concentration versus time curve from zero to 24 hours divided by dose per kilogram body weight
  - AUC_{last}: Area under the concentration-time curve (AUC) from the time of dosing to the time of the last measurable concentration
  - AUC_{last}/D: AUC_{last}/Dose
  - $AUC_{last,norm}$: AUC_{last} divided by dose per kilogram body weight
  - $C_{max,ss,norm}$: Maximum concentration at steady state divided by dose per kilogram body weight
  - T_{max}: The time to maximum observed concentration
  - $t_{1/2}$: Terminal elimination half-life
  - Cl/F: Clearance
  - lambda-z (k_{el}): Elimination rate constant
  - MRT_{last}: Mean Residence Time from the time of dosing to the time of the last measurable concentration
  - Vz/F: Volume of distribution
  - CL_{ss}/F: Clearance at steady state
  - AUC_{t-inf}: AUC extrapolated from t to infinity

- %AUC_{extra}: % AUC extrapolated from the last plasma concentration to infinity
- Points terminal for DNDI-0690: points selected to calculate terminal elimination half-life
- C_{trough} will be derived from the concentration data (Days 2-10 for Part A, days 2-/5 for Part B and days 2/4/7/10 for Part C)
- Accumulation ratios RA (C_{max}) and RA ($AUC_{0-\tau}$)
- Day 10 (Part A) and Day 5 (Part B):
  - Ae_{0-24}: Amount excreted in urine over each collection interval (0 to 24 hours post-dose).
  - Ae%: % of dose excreted in urine over each collection interval (0 to 24 h post-dose).
  - CL_R: Renal clearance

If BID dosing, AUC_{0-10}, AUC_{0-10}/D, $AUC_{0-10h,norm}$ will be added on Day 1 and Day10.

Derived PK parameters will be listed along with the points used to calculate k_{el} (lower, upper and number used) and R^2 adjusted. The derived PK parameters will also be summarised. The descriptive statistics presented will be N, n, arithmetic mean, geometric mean (with the exception of T_{max}), SD, coefficient of variation (CV%), minimum, median and maximum.

Dose Proportionality/Independence (Part A and Part B): For Day 1 and Day 10 (Part A) OR Day 1 and Day 5 (Part B), dose proportionality will be assessed by performing a regression analysis of the log-transformed C_{max}, $AUC_{0-\tau}$ and AUC_{0-inf} (Day 10 for Part A and Day 5 for Part B only) 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.

For Day 10 for Part A and Day 5 for Part B, dose independence will be assessed for t½ and CL/F by performing a regression analysis of the untransformed parameters versus dose with a fixed effect for dose. For each parameter a point estimate and corresponding 95 % CI will be calculated for the slope of the regression line.

Steady-State (Part A and Part B): For each dose level, log-transformed trough concentration levels at pre-dose each day (Day 2 to Day 10 for Part A and Day 2 to Day 5 for Part B) will be subjected to a mixed effects analysis of variance (ANOVA) with study day as a fixed effect and subject as a random effect in order to establish whether and when steady-state has been attained for each dose level. Back-transformed ratios for the comparisons of each consecutive day (*i.e.* Day 3/Day 2) will be presented along with corresponding 90 % CI.

Accumulation (Part A and Part B): For each dose level, log-transformed C_{max} and $AUC_{0-\tau}$ values on Day 1 and day 10 for Part A or Day 1 to Day 5 for Part B will be subjected to an ANOVA with study day as a fixed effect and subject as a random effect. For comparison point estimates and 90 % CI for the difference between Day 10 (Part A) or Day 5 (Part B) and Day 1 will be constructed using the residual mean square error obtained from the ANOVA for each dose level. The point and interval estimates will then be back transformed to give estimates of the ratios of the geometric least squares means and corresponding 90 % CI.

Derived relative PK Data for metabolite M5: The peak areas of M5 will be recorded and a relative comparison of the peak areas of M5 versus DNDI-0960 will be performed for each subject at each time point. The relative abundance of M5 will be expressed as percentage of DNDI-0960 (depending on the data obtained, this may be a relative abundance per time point or via relative AUC comparison via peak area integration over time). Although this is a qualitative analysis of M5, an attempt will be made on a non-quantitative level to classify the abundance of M5 in plasma of subjects compared to DNDI-0960 as major (>10%), minor (2-10%) or negligible (<2%).

10.9.2.5.5 PD Data

N/A.

10.9.2.5.6 Other Data

- mGFR will be calculated following evaluation of plasma clearance of iohexol before and under exposure to DNDI-0690. mGFR data will be descriptively analysed together with their 95% confidence intervals. More details on methodology will be provided in the SAP.
- Exploratory Endpoints:
    - Changes in clinical early renal toxicity markers in urine: Cystatin C (Cys C), Kidney Injury Molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL).
    - Analysis of Holter extracted ECGs for the following parameters: RR, heart rate (HR), PR, QRS, QT, corrected QT interval by Fridericia's formula (QTcF), corrected QT interval by Bazett's formula (QTcB), ΔHR, ΔRR, ΔPR, ΔQRS, ΔQT, ΔQTcF and ΔQTcB.
    - Measurement of drug concentration in dry blood spots for comparison with matching plasma samples
    - Variation of mRNA expression in blood sample before and after exposure to the drug (Transcriptional Profiling)
    - Exploration of metabolism after multiple dose exposure

10.9.3 Sample Size Calculation

The study is exploratory. 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. Based on experience from previous studies of a similar design, a total of 9 subjects are to be enrolled per cohort and a minimum of 6 evaluable subjects per cohort are considered sufficient.

A sufficient number of subjects will be initially screened for enrolment to ensure that the planned sample size is achieved.

11 PRACTICAL CONSIDERATIONS

11.1 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 EMA Guideline INS/GCP/856758/2018[07]. 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's electronic trial master file (eTMF) will be used during the study. Sponsor will be responsible for the setup of eTMF. Sponsor has delegated the maintenance of the Sponsor eTMF to Simbec-Orion. The eTMF will be archived by Sponsor for at least 25 years after the end of the study.

11.2 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:

a. The safety or physical or mental integrity of the subjects of the study.

b. The scientific value of the study.

c. The conduct or management of the study.

d. The quality or safety of any IMP used in the study.

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

Substantial 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.

11.3 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.

11.4 Study Report and Publication Policy

Simbec-Orion will investigate and analyse the data generated with all due speed.

A draft study report will be sent to the Sponsor for review. The Sponsor will forward any comments on the draft study report to the Project Manager within 30 days of receipt. Additional review cycle may be implemented if deemed necessary by the Sponsor. Upon receipt of these comments a final, QA approved report will be issued with all due speed. A copy of the report will be forwarded to the Sponsor.

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

11.5 General Data Protection Regulation (GDPR)

Personal data of the volunteer 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 volunteers' personally identifiable information should be replaced through the use of pseudonymisation.

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

12 REFERENCES

1. Iohexol summary of product characteristics (SmPC), Omnipaque Injection 300mg I/ml solution for injection, GE Healthcare AS, PL 00637/0035, 15 November 2018.
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. DNDI-0690 Investigator' Brochures, Version 3.0, 03 September 2020
6. EU GMP Annex 13: Investigational Medicinal Products, https://www.gmp-compliance.org/guidemgr/files/2009_06_annex13.pdf
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APPENDIX 1: NORMAL RANGES FOR VITAL SIGNS AND ECG PARAMETERS

Vital Sign Parameters	
Pulse Rate	50-100 Beats per minute (bpm)
Systolic Blood pressure	90-140 mmHg
Diastolic Blood pressure	50-90 mmHg
Tympanic Temperature	35.0-37.5 Degree Celsius

ECG Parameters	
Heart Rate (HR)	50-100 bpm
PR Interval	120-220 ms
QRS Width	70-120 ms
QT Interval	$\leq$ 500 ms
QTc Interval- Bazetts' (QTcB)	350-450 ms
QTc Interval -Fredericia's (QTcF)	350-450 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/