CLINICAL STUDY PROTOCOL

A Phase I, Double-blind, Randomised, Single Centre, Parallel-group, Single-dose, Dose-escalation, Placebo-controlled Study of the Safety, Tolerability and Pharmacokinetics of DNDI-0690 after Oral Dosing in Healthy Subjects

Single Oral Dose Escalation Study of DNDI-0690 in Healthy Subjects

Quotient Study Number:	QSC200932
Sponsor Study Number:	DNDi-0690-01
EudraCT Number:	2018-002021-35
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3 Synopsis

Sponsor: DNDi	**Drug Substance:** DNDI-0690	**EudraCT No.:** 2018-002021-35
Title of Study: A Phase I, Double-blind, Randomised, Single Centre, Parallel-group, Single-dose, Dose-escalation, Placebo-controlled Study of the Safety, Tolerability and Pharmacokinetics of DNDI-0690 after Oral Dosing in Healthy Subjects		
Principal Investigator: Sharan Sidhu MBChB, BAO, MRCS, MFPM		
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Objectives:

Primary Objective:

- To assess the safety and tolerability of DNDI-0690 after single oral doses

Secondary Objective:

- To investigate plasma and urinary pharmacokinetics (PK) of DNDI-0690 after single oral doses

Exploratory Objectives:

- To investigate any potential changes to renal toxicity markers in plasma and urine
- To investigate the metabolite profile of DNDI-0690
- To assess the effect of DNDI-0690 on Holter ECG parameters
- To investigate the PK of DNDI-0690 after single doses in the fed versus fasted state in healthy male subjects
- To investigate the PK of DNDI-0690 after single doses in healthy women of non-childbearing potential (WONCBP) versus healthy male subjects

Methodology:

This is a single centre, double-blind, randomised, placebo-controlled, parallel-group, single oral dose, dose-escalation study in healthy male and WONCBP subjects. It is planned to enrol 8 subjects in 8 planned cohorts. Cohorts 1 to 7 will include male subjects. Cohort 8 will include WONCBP subjects. Subjects will be randomly assigned to receive a single oral dose of active investigational medicinal products (IMP) or matching placebo in a sequential escalating manner with a minimum of 7 days between dosing of each cohort.

The planned starting dose for Cohort 1 (Regimen A) will be 10 mg. Doses to be administered in Cohorts 2 to 8 will be determined based on emerging PK and safety data.

Study Design:

Each cohort will follow the same study design. Subjects will be screened for inclusion in the study up to 28 days before dosing. Subjects will be admitted during the morning of the day before dosing (Day -1) for all regimens. Subjects will be dosed in the morning of Day 1 in a randomised, double-blind manner with either DNDI-0690 capsule or matching placebo. Subjects in Cohorts 1 to 6 and 8 will be dosed following a minimum overnight fast of 10 h. Subjects in Cohort 7 will be dosed after a standard high-fat breakfast. Subjects will remain onsite until 72 h post-dose. A follow-up visit will take place 7 to 10 days post-dose to ensure the ongoing wellbeing of the subjects. There will be a minimum of 7 days between dosing of each cohort.

As a safety precaution each cohort will be split into two groups: sentinel (2 subjects) and main (6 subjects). The subjects of the sentinel group (1 subject on active, 1 subject on placebo) will be dosed with an appropriate interval between them as allowed by logistics. After review of the safety data from the 24 h post-dose period, the Principal Investigator (PI), or medically-qualified designees who are familiar with the study protocol and Investigator's Brochure (IB), will decide whether to proceed with dosing the remaining subjects in the main group (5 subjects on active treatment, 1 subject on placebo) at least 24 h after the second sentinel subjects. The first subject of the main group will be dosed no earlier than 24 h after dosing of the second subject in the sentinel group.

Number of Subjects Planned:

It is planned to enrol 8 healthy male subjects in Cohorts 1 to 7 and 8 healthy WONCBP subjects in Cohort 8 (8 planned cohorts, 64 subjects in total). This will ensure data in 6 evaluable subjects per cohort. Subjects withdrawn due to an IMP-related adverse event (AE) will not be replaced. Subjects who are withdrawn for other reasons may be replaced as required by agreement between the PI and Sponsor to ensure sufficient evaluable subjects. Up to 2 additional subjects per cohort may be enrolled into the study. The maximum number of subjects that may be dosed is 80. An evaluable subject is defined as a subject who has sufficient PK and safety data up to 72 h post-dose or was withdrawn prior to 72 h due to an adverse event.

Duration of Study:

Subjects will receive a single dose administration on one occasion. The estimated time from screening until the follow-up visit is approximately up to 6 weeks.

Main Inclusion Criteria:

Healthy males aged 18 to 55 years (Cohorts 1 to 7)

Healthy WONCBP aged 18 to 60 years (Cohort 8)

Body mass index (BMI) 18.0 to 30.1 kg/m^2.

Investigational Medicinal Product, Dose and Mode of Administration:

The following IMPs will be used in this clinical study:

Regimen	IMP Name	Unit Strength[a]	Route of Administration
A to H	DNDI-0690 capsule or matching placebo	10 mg or 100 mg or 200 mg	Oral Administration, Cohorts 1 to 6 and 8: Fasted Cohort 7: Fed

[a] DNDI-0690 capsule strengths are displayed as free drug equivalent.

Pharmacokinetic Assessments:

Blood samples for PK analysis will be collected at regular time points. The plasma concentration data for DNDI-0690 provided by SGS Belgium will be analysed by Quotient Sciences using Phoenix WinNonlin 8.0 or a more recent version (Certara USA, Inc., USA).

Plasma concentration data will be tabulated and plotted for each subject for whom DNDI-0690 concentrations are quantifiable. PK analysis of the concentration time data obtained will be performed using appropriate non-compartmental techniques to obtain estimates of the following PK parameters, where possible:

- **T_{max}:** the elapsed time from dosing at which C_{max} was apparent
- **C_{max}:** the maximum observed concentration
- **$C_{max/D}$:** the maximum observed concentration normalised for dose
- **$C_{max, norm}$:** the maximum observed concentration normalised for dose and body weight
- **$AUC_{(0–24)}$:** area under the curve from 0 time to 24h
- **$AUC_{(0–t)}$:** area under the curve from 0 time to last measurable concentration
- **$AUC_{(0–t), norm}$:** area under the curve from 0 time to last measurable concentration normalised for dose and body weight
- **$AUC_{(0–inf)}$:** area under the curve from 0 time extrapolated to infinity
- **$AUC_{(0–inf)/D}$:** area under the curve from 0 time extrapolated to infinity normalised for dose
- **$AUC_{(0–inf), norm}$:** area under the curve from 0 time extrapolated to infinity normalised for dose and body weight
- **AUC_{t-inf}:** percentage of $AUC_{(0–inf)}$ extrapolated beyond last measured time point
- **Points terminal:** the number of points used to determine the elimination half-life
- **$T_{1/2}$:** the apparent elimination half-life
- **$C_{l/F}$:** apparent plasma clearance after oral administration
- **$V_{z/F}$:** apparent volume of distribution based on area for oral dose
- **MRT:** mean residence time
- **lambda-z:** the slope of the apparent elimination phase

The urine concentration data for DNDI0690 will be analysed for the following PK parameters:

- **Ae:** amount excreted
- **Fe:** fraction of dose excreted
- **CLr:** renal clearance

Safety Assessments:

The safety assessments to be conducted are:

- AEs
- Clinical chemistry, haematology, coagulation and urinalysis assessments, especially renal function parameters.
- Physical Examinations
- 12-lead electrocardiograms (ECGs)
- Vital signs

- Troponin I

Exploratory Assessments

- Holter extracted ECGs
- Urine sampling for Drug-related Renal Injury Exploratory Biomarkers

Statistical Methodology:

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

Dose proportionality will be assessed across dose groups (assuming same prandial status and gender) using the power model. The power model will be applied separately to the ln(AUC) and ln(C_{max}) values. A point estimate and its 90% confidence interval will be calculated for the population mean slope together with the acceptance range.

Further statistical analyses of PK parameters $AUC_{(0-t)}$, $AUC_{(0-inf)}$ and C_{max} will be performed to examine the effect of food in males (comparing fed and fasted cohorts at same dose level) and the effect of gender (comparing male and female cohorts at same dose level).

Sample Size and Power:

The study is exploratory and no formal sample size calculation has been made. Based on experience from previous studies of a similar design, a total of 8 subjects are to be enrolled per cohort and a minimum of 6 evaluable subjects per cohort are considered sufficient.

4 List of Abbreviations

Abbreviation	Definition
ADR	adverse drug reaction
AE	adverse event
ALB	albumin
ALP	alkaline phosphatase
α-GST	alpha-glutathione S-transferase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BCS	Biopharmaceutics Classification System
BID	twice a day
BMI	body mass index
BUN	blood urea nitrogen
CHMP	Committee for Medicinal Products for Human Use
CL	cutaneous leishmaniasis
CLcr	creatinine clearance
CLU	clusterin
CYP	Cytochrome P450
CysC	Cystatin C
DMP	Data Management Plan
EC	ethics committee
ECG	electrocardiogram
EMA	European Medicines Agency
FaSSIF	fasted state simulated intestinal fluid
FDA	US Food and Drug Administration
FeSSIF	fed state simulated intestinal fluid
FIH	First-in-Human
GCP	good clinical practice
GGT	gamma glutamyl transferase
GI	gastrointestinal
GP	general practitioner
HBsAg	hepatitis B surface antigen
HCV Ab	hepatitis C virus antibody
HED	Human Equivalent Dose
HIV	human immunodeficiency virus

HRT	hormone replacement therapy
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonisation
IMP	investigational medicinal product
ISF	Investigator Site File
KIM-1	Kidney Injury Molecule-1
MAD	multiple ascending dose
MedDRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and Healthcare products Regulatory Agency
NGAL	neutrophil gelatinase-associated lipocalin
NOAEL	no observed adverse effect level
OPN	osteopontin
PI	Principal Investigator
PIS	participant information sheet
PK	pharmacokinetic(s)
QA	quality assurance
QC	quality control
QP	Qualified Person
QTcB	corrected QT interval by Bazett's formula
QTcF	corrected QT interval by Fridericia's formula
RAP	Reporting and Analysis Plan
SAE	serious adverse event
SOP	standard operating procedure
SRC	safety review committee
SUSAR	suspected unexpected serious adverse reaction
TEAE	Treatment-emergent adverse event
TPRO	total protein
ULN	upper limit of normal
VL	visceral leishmaniasis
WHO	World Health Organisation
WOCBP	women of childbearing potential
WONCBP	women of non-childbearing potential

5 Background Information

5.1 Introduction

Leishmaniasis is generally seen as one of the most neglected tropical diseases and has strong links with poverty. It comprises a complex vector-borne disease (transmission via sandflies), caused by more than 20 species of the protozoan genus *Leishmania*. Disease manifestations range from localised skin ulcers to fatal systemic disease if untreated [1]. Leishmaniasis is endemic in 101 countries/territories, with 350 million people at risk.

Current treatment options have limitations including toxicity, poor adaptation to field conditions (most are parenteral drugs [sodium stibogluconate, paromomycin, AmBisome®], some require cold chain for transport and storage), and some require long hospitalisations. DNDi's (the sponsor's) priority is to eliminate the use of antimonials and develop a safe, effective, oral, short-course treatment for visceral leishmaniasis (VL) that can be used at any health care level in all foci of the disease .

DNDI-0690 is a 7-substituted nitroimidazooxazine ((7R)-7-({[6-(4-Fluorophenyl) pyridine-3-yl]oxy}methyl)-2-nitro-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine) and under development for the treatment of leishmaniasis.

DNDI-0690 is potent *in vitro* against all laboratory reference *Leishmania* strains and clinical isolates tested with IC_{50} values ranging from 10 nM to 2.5 µM for those causing VL and 0.77 to 4.58 µM for those responsible for causing cutaneous leishmaniasis (CL). DNDI-0690 mechanism of action is not fully understood and likely involves activation via parasitic nitroreductase 2, which has no homologue in humans. Des-Nitro analogues of DNDI-0690 have been shown to be inactive against *Leishmania in vitro*, confirming that the nitrogen dioxide group is essential for activity (further details are available in the Investigator's Brochure [IB] [2]).

In vivo, DNDI-0690 is effective in both the mouse (acute) and hamster (chronic) VL models and against both *L. donovani* and *L. infantum*, at the dose of 12.5 mg/kg once daily (mouse) and 12.5 mg/kg twice daily (hamster) for 5 days, respectively. Pharmacodynamic properties of DNDI-0690 are clearly dose and duration dependent. This relationship can be considered for human dose prediction and adjustment.

The information from the non-clinical pharmacology studies conducted with DNDI-0690 thus suggests that this new chemical entity will effectively inhibit *Leishmania* parasites.

DNDI-0690 is intended to be used as oral treatment for VL with potential for the cutaneous form of the disease, CL. The present protocol describes the first-in-human (FIH) study with DNDI-0690.

5.2 Investigational Medicinal Product(s)

The following investigational medicinal products (IMPs) will be used in this clinical study (Table 1).

Table 1 Investigational Medicinal Products

Regimen	IMP Name	Unit Strength[a]	Route of Administration
A to H	DNDI-0690 capsule or matching placebo	10 mg or 100 mg or 200 mg	Oral Administration, Cohorts 1 to 6 and 8: Fasted Cohort 7: Fed

[a] DNDI-0690 capsule strengths are displayed as free drug equivalent.

The IMP, DNDI-0690, is an un-licensed medicinal product for use only in the proposed clinical trial.

Only subjects enrolled in the study may receive study treatment and only authorised site staff may supply or administer study treatment. All study treatments will be stored in a secure, environmentally-controlled, and monitored (manual or automated) area in accordance with the labelled storage conditions with access limited to the investigator and authorised site staff.

All used and unused IMPs will be reconciled, returned to sponsor or destroyed in accordance with the study-specific quality agreement and technical addendum.

5.2.1 Supply, Packaging, Labelling and Storage of Investigational Medicinal Products

The sponsor will ensure that all IMPs are characterised and manufactured in accordance with any applicable requirements of Good Manufacturing Practice and regulatory requirements.

After the trial protocol was approved by the ethics committee (EC) and regulatory authorities, the sponsor will supply the investigator and trial sites with all trial medication, together with all relevant documentation including a description of the storage conditions.

DNDI-0690 capsules (active and placebo) should be stored in the original container between 15°C and 25°C. The IMP should not be used after the expiry date indicated on the label.

Each dose strength (10 mg, 100 mg, 200 mg and matching placebo) is packaged into 100 mL HDPE bottles with induction seal closure. A fill count of 30 capsules per bottle has been adopted. The number of capsules to be administered will depend on the dose cohort. The Manufacturing Team (or other trained staff who are not involved in the clinical conduct) at the trial site will prepare the capsules in HDPE containers for each subject according to the randomisation list.

The sponsor will maintain a complete record of batch numbers and expiry dates of all IMP as well as the labels of all IMP in the trial master file.

The investigator will maintain, amongst other documents, a record of the batch numbers and expiry dates of all IMP as well as the labels of the IMP received at the trial site in the investigator site file (ISF).

5.3 Previous Study Findings

5.3.1 Non-clinical Findings

5.3.1.1 Safety Pharmacology

No safety concerns were identified for respiratory functions and central nervous system following administration of DNDI-0690. Potential to delay cardiac ventricular repolarization (determined *in vitro* - human ether-à-gogo related gene [hERG] and panel of ion channels- as well as *in vivo* in telemetered monkeys) was assessed and results showed that no pharmacological effects on cardiovascular parameters are expected at the doses intended to be administered. Indeed, DNDI-0690 free plasma concentration of at least 0.63 µM was reached in the telemetry study without raising any signal, while the maximum anticipated C_{max} in humans at the predicted efficacious dose will be 0.10 µM.

5.3.1.2 Non-clinical Pharmacokinetics and Human Pharmacokinetic Prediction

Available *in vitro* absorption, distribution, metabolism, and excretion (ADME) and pharmacokinetic (PK) data indicate that at least the unbound concentrations of DNDI-0690 are readily taken up into the tissues of interest such as liver, spleen or bone marrow, ie, main target tissues hosting the parasite *Leishmania*.

This assumption is supported by the exposure data in mouse and hamster: in both models, threshold of significant efficacy seems to be correlated to a similar minimum unbound drug concentration at steady state.

DNDI-0690 is a low clearance compound in all species (human, monkey, minipig and rat) and has a medium clearance in dog microsomes. The parent compound is only slowly metabolised and very low levels of metabolites were detected *in vitro* (human, dog, monkey and rat). The same is true for *in vivo* studies in rat and monkey. Importantly, given the very low intrinsic clearance in human hepatocytes, there is a high likelihood that DNDI-0690 is also a low clearance drug in humans. Extensive PK modelling was performed to not only predict human plasma clearance (CL_p) but also volume of distribution at steady state (V_{ss}), half-life ($t_{1/2}$) and bioavailability (F). A brief summary is given as follows and is based on modelling work reported in several key publications [3],[4],[5].

The prediction of clearance was performed via two methods [3], ie inter-species allometric scaling using *in vivo* clearance from pre-clinical species and, independent of this, intra-species scaling using *in vitro* intrinsic clearance in hepatocyte (with and without correction for plasma protein binding) of all available species including human in combination with the physiologically based PK-based well-stirred model which allows scaling to an intact human liver. The latter approach technically predicts “hepatic clearance” only. Importantly, both approaches resulted in similar clearance predictions indicating that liver metabolism appears to be the major driving force behind DNDI-0690 total plasma clearance (CL_p) with a CL_p value anticipated to be less than 10% of liver blood flow).

The human V_{ss} was also predicted using two independent methods [3], ie inter-species allometric scaling of V_{ss} without correction for plasma f_u or the proportionality method where human V_{ss} is predicted via single-species in animals V_{ss} with correction of plasma f_u. Likely due to the low inter-species differences of plasma protein binding all methods delivered highly similar results with the mean of all data predicting a human V_{ss} of ~0.7 L/kg (medium volume distribution which is essentially the volume of body water).

Half-life ($t_{1/2}$) is a resulting parameter only, ie it is a result of CL_p and V_{ss} and, most often, it also depends on further definitions (eg terminal half-life, dominant half-life under the major AUC). Thus, predictions were based on either scaling of terminal half-life data from animals via direct species correlation method [3] or via calculation of resulting $t_{1/2}$ from predicted CL_p and V_{ss}. The latter approach is more reflective of a true operational $t_{1/2}$ under steady state conditions [5]. Despite the different methods, the resulting simulated half-lives were in good agreement and final prediction using the mean of all results indicated that the relevant human $t_{1/2}$ following multiple administrations and under steady state conditions (as described [5]) is in the range of 7 h.

Finally, the bioavailability is predicted to be “medium” at ~30% in a conservative scenario based on direct comparison with data in rat and monkey which allows at least a binning prediction for bioavailability [6].

These human predicted PK parameters (Table 2) were used to further calculate potential plasma exposures at steady state.

Metabolite identification studies were conducted in human, dog, monkey and rat liver microsomes. The results suggested that DNDI-0690 is mainly metabolised via oxygenation in rat, dog, and human liver microsomes as only one metabolite (M1) was detected in these species, representing less than 0.4% of parent. In monkey liver microsomes, M1 was again detected, with two other metabolites (M2 and M3), for a total peak area ratio of less than 3.0% of total mass spectrometry peak area. Consequently all human metabolites detected so far *in vitro* are covered by toxicology species used as part of the regulatory preclinical package (rat and monkey).

The time-dependent inhibition of cytochrome P450 (CYP) by DNDI-0690 was investigated using individual CYP isoforms. Based on the current understanding of plasma concentrations needed to achieve efficacy, clinically relevant liability for drug-drug interaction due to CYP inhibition with DNDI-0690 is unlikely.

Human PK parameters prediction and animal data suggest that DNDI-0690 bioavailability should not be limited by first-pass metabolism. Bioavailability is likely dissolution-rate limited (nano-suspensions improved exposures), consequently a sub-proportional increase of exposure with dose may be expected. Using the overall predicted human PK parameters in combination with animal efficacy data from hamster (best case) or mouse (worst case) the total efficacious daily human dose is estimated to be between 40 and 110 mg for a 50 kg human being (corresponding to weight reasonably expected in adult patients with VL; for expected exposures at these predicted doses see Table 2.

All human metabolites detected *in vitro* are covered by toxicology species rat and cynomolgus monkey. Metabolites detected *in vivo* were at low or trace levels, thus not likely to be of further concern.

This suggests no metabolite needs to be quantified in the FIH clinical trial. However, qualitative metabolite identification in selected human plasma samples may be considered to confirm the data in animals.

5.3.1.3 Toxicology Findings

There was no noteworthy finding in rat repeat dose toxicology studies. A no observed adverse effect level (NOAEL) of 1000 mg/kg/day after a 4 week treatment was established, corresponding to an Area Under the Curve from 0 to 24 hours ($AUC_{(0-24)}$) at steady state of 180 and 307 h·µg/mL in males and females, respectively. In the cynomolgus monkey, kidneys and testes were identified as target organs and findings were clearly time and dose dependent. In particular, renal toxicity was observed during the 4-week pivotal study at a dose of 100 mg/kg/day and above, but full recovery was demonstrated after 7 weeks, as measured in the highest dose group (400 mg/kg/day). Microscopic changes were observed in the testes of some monkeys treated for 4 weeks at 100 and 400 mg/kg/day. These changes were mainly characterized by slight atrophy of the tubular seminiferous epithelium and sometimes by a consequent decrease in tubular size, suggesting a direct effect on the cells rather than an indirect hormonal effect. Only a partial recovery was achieved for this organ after a 7 week recovery period, since one animal treated at 400 mg/kg still showed incomplete presence of maturing cells. Consequently, the dose of 20 mg/kg/day given for 4 weeks is considered as the NOAEL in the cynomolgus monkey that is the most sensitive species, and corresponds to an AUC_{0-24} at steady state of 101 and 111 h·µg/mL and an Cmax of 8.03 and 9.03 µg/mL in males and females, respectively.

5.3.1.4 Phototoxicity

As DNDI-0690 does not absorb light within the range of natural sunlight (290-700 nm λmax 255 nm), no further photosafety assessment has been conducted.

5.3.1.5 Non-clinical Data Summary

The non-clinical safety studies indicate that DNDI-0690 is expected to be safe to administer to healthy subjects by the oral route at a starting dose of 0.645 mg/kg or 45 mg per 70 kg subject (calculated based on a NOAEL of 20 mg/kg in male and female monkeys and applying a safety margin of 10, see Section 6.2) and to a maximum dose equivalent to an AUC_{0-24} and C_{max} values of 101 and 111 h·µg/mL and 8.03 and 9.03 µg/mL, based on data in male and female monkeys respectively. The starting dose in this trial is 10 mg below the maximum safe starting dose of 45 mg.

5.3.2 Clinical Findings

DNDI-0690 has not been administered to humans previously. This study will be the first time DNDI-0690 will be administered in humans (FIH).

6 Rationale

6.1 Study Rationale

DNDI-0690 is under development for treatment of Leishmaniasis. The present protocol constitutes a FIH study and all applicable precautions to ensure the safety of the subjects participating in the study will be taken.

In this trial, first data of PK and safety of single ascending oral doses of DNDI-0690 in humans will be assessed to evaluate further development of this compound.

DNDI-0690 is expected to have a relatively short half-life in humans (7.4 h) [2]. This may result in the need for twice a day (BID) dosing in the future multiple ascending dose (MAD) study, and consequently dosing of subjects in a non-fasted state. Knowledge of the effect of food on both exposure and tolerability of DNDI-0690 will support the design of the next clinical study by ensuring a better prediction of exposure and consequently better safety monitoring.

As women are part of the VL patient population, it is important for the development program to confirm if the exposure to DNDI-0690, the safety and tolerability of the drug are similar in women compared to men. If they are confirmed to be similar, this would support the inclusion of male and female subjects in the next clinical study and will help to ensure a better prediction of exposure and consequently better safety monitoring.

This trial is part of a clinical development program, and further trials may be conducted.

6.2 Dose Rationale

The starting dose for this dose-escalation study was selected on the basis of the results of the 28-day toxicity studies in the rat and non-human primate (Cynomolgus), which indicated a NOAEL at 1000 mg/kg/day in rats and 20 mg/kg/day in non-human primates.

The Human Equivalent Dose (HED) would be 161 mg/kg and 6.45 mg/kg, calculated from NOAEL in rat and Cynomolgus (28-day toxicity studies) respectively, according to US Food and Drug Administration (FDA) Guidance for Industry [7], equivalent to 11.2 g and 451 mg for a 70 kg subject.

Considering the lowest of these HED values and a minimum safety margin of 10 as recommended by the FDA guidance above, a starting dose of 0.645 mg/kg was considered (ie, 45 mg for a 70 kg subject). This dose corresponds to the lowest estimate of the potential efficacious dose. In order to initiate this study at a dose lower than a potentially efficacious dose, the starting dose is to be 10 mg for a 70 kg subject.

The dose levels will be reviewed and can be altered based on emerging data. Dose escalation will only occur if the criteria listed in Section 8.2.1 is not met. If this criteria is met, or if it is deemed necessary, the dose selected for the subsequent cohort may be lower than the last. Dose escalation between cohorts will be limited to a maximal 5-fold increase for the first 3 cohorts and a maximal 3-fold increase thereafter, depending on safety and tolerability data and predicted exposure (both C_{max} and AUC). If mild or moderate adverse events (AEs) occur at a particular dose level, that dose level may be repeated, with the aim of further exploring the relationship between dose/exposure and AEs. If, in the judgement of the safety review committee (SRC), it would not be reasonable to expose more subjects to the level of discomfort experienced by those who have already received the dose, the next scheduled dose may be reduced. The reduction may be either to one of the dose levels that has already been given, or to an intermediate level that has not previously been given; in either case, the aim is to learn more about the relationship between AEs and dose (or plasma concentration) of DNDI-0690.

The exposure caps listed in Section 8.2.1 would allow for the predicted efficacious dose to be slightly exceeded. This is considered reasonable as the efficacious dose cannot be accurately confirmed at this stage, to allow the evaluation of the safety and PK of DNDI-0690 slightly beyond the predicted margin, see Table 2.

At high doses in non-clinical studies the bioavailability of DNDI-0690 decreased due to low solubility, hence lower absorption at high doses in preclinical species. In humans, DNDI-0690 absorption is also expected to decrease with dose, and DNDI-0690 is expected to show sub-proportional exposure increases at least at the higher dose levels.

There is no data on interactions with food once DNDI-0690 is administered in humans. Healthy subjects enrolled into Cohorts 1 to 6 and 8 in this study will therefore receive DNDI-0690 in the morning, after a fasting period of at least 10 h. Cohort 7 will include dosing after a high-fat breakfast (provided at least 30 min before dosing), to allow for a clinical assessment of interaction with food.

DNDI-0690 is a Biopharmaceutics Classification System (BCS) class II drug with poor aqueous solubility and good bioavailability. The drug is poorly soluble in gastrointestinal (GI) fluid, leading to saturation of the dissolution process. At some point, no more drug can dissolve into the GI fluid to be available for absorption. This could explain a plateau in drug exposure where increasing the dose is not proportionally increasing the exposure. Dissolution of active product ingredient in fed state simulated intestinal fluid (FeSSIF) media (pH 5.0), mimicking the fed condition, shows an increased dissolution up to 0.011 mg/mL after 4 h while fasted state simulated intestinal fluid (FaSSIF) media (pH 6.5), mimicking fasted condition, allows dissolution to reach 0.003 mg/mL [8]. The dose selected for the fed cohort will be approximately 3 times lower than the maximum dose shown to be well tolerated in the fasted state.

Exploring the dosing of DNDI-0690 in fed state will provide the following information:

- Firstly, dosing in fed state could potentially increase the absorption of the drug and thus could result in increased overall exposure, compared to fasted conditions, in order to reach the predicted exposure required for efficacy.
- Secondly, because the drug is predicted to have a relatively short half-life, this may result in the need for a BID dosing in the future MAD study, and consequently dosing of volunteers in a non-fasted state (afternoon administration).

Knowledge of the effect of food on both exposure and tolerability of DNDI-0690 will support the design of the next clinical study by ensuring a better prediction of exposure and consequently better safety monitoring.

Based on the predicted human PK parameters and considering the minimal unbound concentrations at steady state ($C_{ss,min,u}$) needed for efficacy in the mouse and hamster leishmaniasis models, the human doses were calculated (standard clinical PK equations [6]) and $k_{abs}>>k_{el}$) to reach the same $C_{ss,min,u}$ as in mouse (worst case) and hamster (best case). The resulting doses suggest that efficacy in humans may be achieved between 40 mg (based on hamster) and 110 mg/day (based on mouse) for a 50 kg patient with VL, equivalent to 56 mg and 154 mg/day for a 70 kg body weight subject. The underlying exposures at these doses are summarized in Table 2 below and put into context with projected exposures at starting dose and at highest dose.

Table 2 also serves as an indicator of the potential therapeutic index of DNDI-0690. For example, the projected exposure at the highest predicted efficacious dose based on total AUC provides a safety margin of 8-fold over the stopping rule exposure (essentially the same if C_{max} is considered).

Table 2 Prediction of Exposure in Humans

DNDI-0690	Dose/subject (mg)	Est Human C_{max} (µg/mL)	Est. Human AUC_{0-24} (h*µg/mL)
First cohort dose	10	0.0958	0.913
Lowest Predicted efficacious dose	40[a] (56[b])	0.383	3.65
Highest Predicted efficacious dose	110[a] (154[b])	1.054	10.0
Example of a higher dose	500	4.790[c]	45.7[c]

[a] Dose is based on a 50 kg visceral leishmaniasis (VL)-patient
[b] Dose adjusted based on a 70 kg healthy volunteer
[c] Predicted AUC_{0-24} does not reach AUC_{0-24} based on stopping rule (100 h*µg/mL); Predicted C_{max} does not reach C_{max} defined as stopping rule (8.0 µg/mL)

6.3 Population Rationale

The purpose of this study is to show a difference between the applied conditions, and therefore it is an advantage to minimise variability.

Healthy male and females of non-childbearing potential volunteers will be included in the trial. As no information of reproductive toxicity in female subjects is available to date, women of childbearing potential (WOCBP) will be excluded from participating in the study. As women are part of the VL patient population, it is important for the development program to confirm if the exposure and the tolerability of DNDI-0690 in healthy women are no different from those in healthy men. Following completion of this study if they are confirmed as similar, this would support the inclusion of male and female subjects in the future studies and will help to ensure a better prediction of exposure and consequently better safety monitoring.

In order to facilitate recruitment of a sufficient number of WONCBP (which includes post-menopausal women) the age range for females enrolled in the trial will be 18 to 60 years.

In order to avoid any interaction with other medication, no prescribed or over-the-counter drug or vitamins/herbal remedies (eg 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 co-medication will be allowed except up to 4 g of paracetamol in 24 h and those deemed necessary by the Investigator to treat AEs, see also Section 9.3. Based on observations in non-clinical studies, a parallel-group design is considered adequate for this study.

Subjects included will be male in the age group 18 to 55 or women of non-childbearing potential (WONCBP), in the age group 18 to 60 years, of normal weight, non-smokers and have no history of alcohol or drug abuse. The latter criteria are proposed to avoid interaction on drug metabolism and to avoid non-compliance as per European Medicines Agency (EMA) guidance.

6.4 Risks and Benefits

In non-clinical studies, no safety concerns were identified for respiratory function and central nervous system after administration of DNDI-0690. Furthermore, no pharmacological effects on cardiovascular parameters are expected at the doses to be administered in this trial.

Kidney was identified as potential target organ for toxicity in the 28 day DNDI-0690 administration in cynomolgus monkeys. In this study, only single doses will be given, thus, the risk for renal toxicity is considered to be low.

Nevertheless, special attention will be paid to any changes in renal function (creatinine, blood urea nitrogen [BUN], creatinine clearance (CLcr); see Appendix 1). Clinical early renal toxicity biomarkers in plasma and urine may be investigated if changers in renal function are observed. Furthermore, subjects with impaired renal function will be excluded (Section 9.3), and rules for no further dose escalation based on renal function markers are implemented (Section 8.2.1).

According to noteworthy changes observed in DNDI-0690 pivotal toxicity study in cynomolgus monkeys (no significant change was observed in the rat), some biochemistry changes may be anticipated in humans and thus will be monitored accordingly during the clinical trial. Total serum bilirubin, alkaline phosphatase (ALP), glutamate dehydrogenase, cholesterol, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels will be especially monitored to assess any effect on liver function.

Changes in the heart in a GLP 4-week study in cynomolgus monkeys were observed, but only in the 2 female monkeys that died at Day 23 and 29. Histological findings suggested that these changes were secondary to agony. The cardiac safety of the subjects will be carefully monitored by holter extracted electrocardiograms (ECGs) as well as safety ECGs and measurement of serum Troponin I concentrations as a cardiac safety marker.

In the 4 week toxicity study in monkeys, effects on the testes (atrophy of the tubular seminiferous epithelium, decrease in tubular size) were observed at 100 and 400 mg/kg per day doses after 28 days of treatment. The overall exposure at which these effects were observed are markedly higher than the expected exposures in humans (after single doses) therefore, the risk for humans is considered to be low in this study. Nevertheless, male study subjects should be informed of potential effects of DNDI-0690 on human fertility, as suggested by the morphological changes observed in the testes of some non-human primates treated in the 4-week toxicology study.

Non-clinical findings for Pretomanid ([S]-PA-824) and Delamanid (Deltyba®, OPC-67683 from Otsuka Pharmaceuticals), two compounds with a high degree of structural similarity to DNDI-0690, potentially reflect some “class effect” as the testis and kidney were also identified as target organs in non-clinical studies.

Administration of Pretomanid or Delamanid in human healthy subjects did not lead to significant side effects and were well tolerated. These data suggest that this class of compound is relatively well tolerated and that clinical monitoring of heart and renal function, together with the other standard measures should ensure the safety of the volunteer subjects during this Single Ascending Dose (SAD) FIH study.

There is no data on interactions with drugs once DNDI-0690 is administered in humans. Whilst no drug to drug reactions are foreseen, the use of prescription medicine is restricted/prohibited except those used to treat AEs as judged by the Investigator or delegate, see Sections 9.3 and 11.4.

No data on reproductive toxicity and embryo-foetal developmental toxicity are available to date. Therefore, WOCBP will be excluded from participating in this study.

Acute toxicity with single DNDI-0690 dosing is not anticipated to pose a risk of concern to healthy volunteers.

Collecting blood samples from a vein may cause pain, swelling, bruising, light-headedness, fainting, and very rarely, clot formation, nerve damage and/or infection at the venepuncture/cannulation site.

During cannulation, more than one attempt may be needed to insert the cannula in a vein of a subject and it is possible that bruising and/or inflammation may be experienced at the site of cannulation.

Electrocardiogram stickers on the subjects' chests and limbs may cause some local irritation and may be uncomfortable to remove but subjects will be closely monitored to ensure any local irritation does not persist.

There is no benefit to the subjects from taking part in this study. The development of a product to improve the treatment of Leishmaniasis will be of benefit to patients with the disease.

The overall risk benefit balance is therefore considered to be acceptable.

7 Objectives and Endpoints

7.1 Objectives

7.1.1 Primary Objective

- To assess the safety and tolerability of DNDI-0690 after single oral doses

7.1.2 Secondary Objective

- To investigate plasma and urinary PK of DNDI-0690 after single oral doses

7.1.3 Exploratory Objectives

- To investigate any potential changes to renal toxicity markers in plasma and urine.
- To investigate the metabolite profile of DNDI-0690.
- To assess the effect of DNDI-0690 on Holter ECG parameters.
- To investigate the PK of DNDI-0690 after singles doses in the fed versus fasted state in healthy male subjects
- To investigate the PK of DNDI-0690 after single doses in healthy WONCBP versus healthy male subjects

7.2 Endpoints

7.2.1 Primary Endpoints

- Safety parameters (changes in vital signs, ECG, safety laboratory parameters with special focus on renal function parameters serum creatinine, BUN, and CLcr) and treatment-emergent adverse events (TEAEs).

7.2.2 Secondary Endpoints

- Measurement of the PK parameters $AUC_{0\text{-}inf}$, $C_{max,}$ $AUC_{0\text{-}inf/D}$, $C_{max/D}$, $AUC_{0\text{-}24}$, $AUC_{0\text{-}inf,norm}$, $AUC_{0\text{-}t}$, $AUC_{0\text{-}t,norm}$, $C_{max,norm}$, T_{max}, T½, MRT, $AUC_{t\text{-}inf}$, $V_{z/F}$, $C_{l/F}$, lambda-z and points terminal for DNDI-0690.
- Measurement of the urine PK parameters Ae, Fe and CLr for DNDI-0690.

7.2.3 Exploratory Endpoints

- Changes in clinical early renal toxicity markers in urine: alpha-glutathione S-transferase (α-GST), clusterin (CLU), Cystatin C (CysC), Kidney Injury Molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), osteopontin (OPN), albumin (ALB) and total protein (TPRO).
- Identification of DNDI-0690 metabolite profile (to be reported separately)
- 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.
- Comparison of the PK parameters $AUC_{(0\text{-}t)}$, $AUC_{(0\text{-}inf)}$ and C_{max} of DNDI-0690 in the fed state versus fasted state in male subjects
- Comparison of the PK parameters $AUC_{(0\text{-}t)}$, $AUC_{(0\text{-}inf)}$ and C_{max} of DNDI-0690 in WONCBP subjects versus male subjects

8 Study Design

8.1 Study Plan

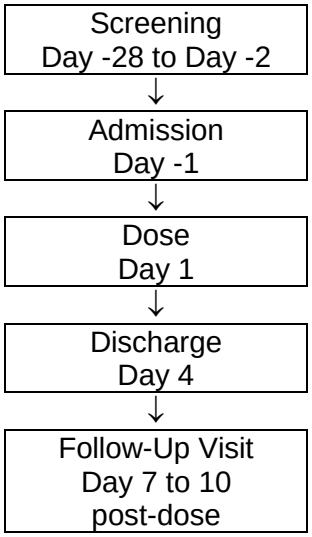
This is a single centre, double-blind, randomised, placebo-controlled, parallel-group, single oral dose, dose-escalation study in healthy male and WONCBP subjects. It is planned to enrol 8 subjects in 8 planned cohorts. Cohorts 1 to 7 will include male subjects. Cohort 8 will include WONCBP subjects. Subjects will be randomly assigned to receive a single oral dose of active IMP or matching placebo in a sequential escalating manner with a minimum of 7 days dosing of each cohort.

The planned starting dose for Cohort 1 (Regimen A) will be 10 mg. Doses to be administered in Cohorts 2 to 8 will be determined based on emerging PK and safety data, see Section 8.2.1. Details of the IMPs are provided in Section 5.2.

Each cohort will follow the same study design (Figure 1). Subjects will be screened for inclusion in the study up to 28 days before dosing. Subjects will be admitted during the morning of the day before dosing (Day -1) for all regimens. Subjects will be dosed in the morning of Day 1 in a randomised, double-blind manner with either DNDI-0690 capsule or matching placebo. Subjects in Cohorts 1 to 6 and 8 will be dosed following a minimum overnight fast of 10 h. Subjects in Cohort 7 will be dosed after a standard high-fat breakfast. Subjects will remain onsite until 72 h post-dose. A follow-up visit will take place 7 to 10 days post-dose to ensure the ongoing wellbeing of the subjects. There will be a minimum of 7 days between dosing of each cohort.

As a safety precaution each cohort will be split into two groups: sentinel (2 subjects) and main (6 subjects). The subjects of the sentinel group (1 subject on active, 1 subject on placebo) will be dosed with an appropriate interval between them as allowed by logistics. After review of the safety data from the 24 h post-dose period, the Principal Investigator (PI), or medically-qualified designees who are familiar with the study protocol and Investigator's Brochure (IB), will decide whether to proceed with dosing the remaining subjects in the main group (5 subjects on active treatment, 1 subject on placebo) at least 24 h after the second sentinel subject. The first subject of the main group will be dosed no earlier than 24 h after dosing of the second subject in the sentinel group.

Figure 1 Study Sequence



Dose selection for each Regimen B to H, will be made upon review of PK and safety data from preceding cohorts, see Section 8.2.1.

Following the administration of the first 7 regimens (A to G), there will be an interim data review during which the PK and safety data will be reviewed. The decision to proceed with the next regimen will be dependent on the PK and safety data from previous cohorts, see Section 8.2.1.

Full details of the interim data reviews are provided in Section 8.2.

8.2 Criteria for In-Study Decisions

In-study decisions will be made by at a minimum the investigator, the sponsor's project manager and a PK expert and/or the sponsor's medical responsible where appropriate.

8.2.1 Criteria for Dose Escalation

Progression to the next dose group will be permitted after review of safety and PK data suggests that it is safe to do so. Dose increases will only be made after a complete review of all data collected from the previous dose group by a SRC as defined in the SRC charter. The SRC will always comprise at a minimum the investigator, the sponsor's medical responsible and a PK expert where appropriate. Full description of the SRC activities will be part of a SRC Charter implemented before the dosing of the first volunteer.

Each dose cohort will be split into 2 groups, a sentinel group (1 subject on active treatment, 1 subject on placebo), and a main group (5 subjects on active treatment, 1 on placebo). The decision to proceed with the main group will be made by the investigator, based on safety data until 24 h post-dose of the second sentinel subject (see also Section 8.5 for stopping criteria). The investigator will inform the sponsor of any safety concerns.

For dose escalation (or reduction, see below) to proceed, data must be available from a minimum of 6 subjects who have completed the planned safety assessments up to 48 h after dosing and the planned PK assessments up to 48 h after dosing to ensure at least 4 subjects had received active IMP.

The following data are required:

- AEs (mandatory)
- Vital signs
- Safety laboratory, specifically renal function parameters
- ECG
- Physical examinations
- Plasma concentrations of DNDI-0690
- Interim PK parameter estimations: AUC and C_{max}

The time points for Troponin I sample collection may be altered based on emerging data from previous cohorts.

Data provided by Quotient Sciences will be provided to the SRC in accordance with the Quotient Sciences standard operating procedure (SOP) on interim dose decision making and dose escalation and as described in the SRC Charter. The decision will be documented and signed by the PI as per Quotient Sciences current SOP and by the DNDi representative as described in the SRC Charter. Evidence of the decision will be retained in the ISF.

Dose escalation will not occur if:

- A serious adverse reaction (ie, a serious adverse event [SAE] considered at least possibly related to the IMP administration) occurs in one subject.

  Or

- Severe non-serious adverse reactions (ie, 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 pre-ceding dose group experience any of the following:

  - ALT or AST >3x upper limit of normal (ULN) and considered IMP related.

  - Decrease in CLcr below 80 mL/min and judged as clinically significant by the investigator (with reference to the change from baseline value) and considered IMP related.

- an absolute increase in serum creatinine of ≥0.3 mg/dL or ≥ 26.4 µmol/L which is judged as clinically significant and considered IMP related.

Or

- If one or more subjects in the preceding dose group experience:

  - an increase in Troponin I concentration > ULN which is considered clinically significant by the investigator (with reference to the change from baseline value) and considered IMP related.

  - an increase in QTcF value of >60 msec from baseline, confirmed by repeat triplicate measurements or an absolute increase in QTcF >500 msec, confirmed by repeat measurement and considered IMP related.

  Or

- if the dose in a single subject is anticipated to exceed an AUC_{0-24} of ≥100 µg×h/mL. These limits are based on the exposure observed at the 20 mg/kg in cynomolgus monkeys (NOAEL; AUC_{0-24} 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 will be limited to a maximal 5-fold increase for the first 3 cohorts and a maximal 3-fold increase thereafter, depending on safety and tolerability data and predicted exposure (both AUC and C_{max}).

If this criteria is met, or if it is deemed necessary, the dose selected for the subsequent cohort may be lower than the last as long as the study stopping criteria (Section 8.5) has not been met. The reduced dose level may be an intermediate dose level to those already assessed or may be repetition of a previously administered dose level with the aim to gather further data for that specific dose level. If mild or moderate AEs occur at a particular dose level, that dose level may be repeated, with the aim of further exploring the relationship between dose/exposure and AE. Any decision to investigate an intermediate or reduced dose level will be fully documented.

Following review of safety and PK data and after completion of Cohort 5, the SRC has determined that fed dosing in Cohort 7 and the inclusion of WONCBP in Cohort 8 are required in order to further explore the PK and safety profile of DNDI-0690. The dose in Cohort 7 will not exceed the 1/3 of the maximum tolerated dose in fasted state. The dose in Cohort 8 will not exceed the maximum tolerated dose in males.

This decision will be made based on PK and safety data from preceding cohorts, as outlined above.

8.3 Subject Withdrawal

If a subject wishes to leave the study at any time, they will be permitted to do so. Every reasonable effort will be made by Quotient Sciences to complete a final assessment/discharge procedures. Quotient Sciences will advise the sponsor of the withdrawal of any subject from the study.

Early withdrawal is defined as the date of the decision to withdraw the subject from the study. Subject completion is defined as the date of the last procedure conducted or last contact (ie phone call) for that subject.

If a subject requests to leave the clinical unit earlier than the planned discharge time eg due to unforeseen personal circumstances, but aims to return to the clinical unit to complete the study, this will be documented as a subject self-discharge and a protocol deviation. The subject must complete the planned assessments/discharge procedures before discharge from the clinical unit and will return for the next assessments, as planned.

Subjects will be withdrawn from the study drug(s) for the following reasons:

- Experiencing a serious or severe AE including but not limited to:
    - QTcF interval of >500 msec or increase in QTcF interval of >60 msec from baseline (confirmed following a repeat ECG after 10 min in resting position)
    - ALT concentration >3 × the upper limit of the reference range (confirmed following a repeat ALT blood test)
- Experiencing a 25% decrease in CLcr
- Pregnancy
- Concurrent illness or requirement for prohibited medication
- At the discretion of the investigator

Subjects will be withdrawn from the study for the following reasons:

- Termination of the study
- Upon the subject's request (withdrawal of consent)
- Significant deviation from the protocol

For the purpose of QTcF withdrawal criteria, baseline will be considered as the average of the pre-dose Day 1 triplicate measurement. For the purpose of CLcr and ALT withdrawal criteria, baseline will be considered as the admission Day -1 measurement.

For a subject who withdraws because of an IMP-related AE, every effort will be made to ensure the subject completes follow-up procedures. Any subject withdrawn or discontinuing the study prematurely because of an IMP-related AE or termination of the study will be considered to have completed the study, and will not be replaced.

Subjects withdrawing for other reasons may be replaced at the discretion of the investigator and sponsor.

8.4 Subject Replacement

Up to 16 (2 per cohort) replacement subjects may be enrolled into the study. The maximum number of subjects that may be dosed is 80.

Subjects withdrawn due to a IMP-related AE or termination of the study will be considered to have completed the study, and will not be replaced.

Subjects who are withdrawn for other reasons may be replaced at the discretion of the investigator and sponsor to ensure sufficient evaluable subjects.

Replacement subjects enrolled will be dosed with the same regimen as the subject they replace.

An evaluable subject is defined as a subject who has sufficient PK and safety data up to 72 h post-dose or was withdrawn prior to 72 h due to an adverse event.

8.5 Stopping Criteria

The study will be halted, and the risk to other subjects evaluated if any of the following criteria are met:

- A serious adverse reaction (ie, a SAE considered at least possibly related to the IMP administration) in one subject.
- Severe non-serious adverse reactions (ie, severe non-serious AE considered as, at least possibly related to the IMP administration) in two subjects in the same cohort, independent of within or not within the same system organ class.

Relatedness will be determined by the investigator. For SAEs the Sponsor will make a parallel assessment; please refer to Section 14.5.2.

If the study is halted, a temporary halt will be submitted to the Medicines and Healthcare products Regulatory Agency (MHRA) and EC in the form of a substantial amendment. The study may be resumed or terminated; however, it will not be resumed until a further substantial amendment to resume the study is submitted and approved by MHRA and EC.

8.6 Study Termination

After the start of protocol activities but prior to the commencement of dosing, the study may be terminated by the sponsor and investigator without consultation with the MHRA and EC. The end of the trial must be notified to the MHRA and EC immediately and at the latest within 15 days after the study is terminated, clearly explaining the reasons. A description of follow-up measures taken for safety reasons if applicable, will also be provided.

If the study is abandoned prior to commencement of any protocol activities, the PI or sponsor must notify the EC and MHRA by letter outlining the reasons for abandonment of the trial.

Once exposure to dosing has begun, the study will be completed as planned unless the following criteria are satisfied that require a temporary halt or early termination of the study.

- The occurrence of serious or severe adverse event(s), as defined in Section 8.5, if considered to be related to the IMP, as defined in Section 14.5.

- New information regarding the safety of the IMP that indicates a change in the risk/benefit profile for the compound, such that the risk/benefit is no longer acceptable for subjects participating in the study.
- Significant violation of Good Clinical Practice (GCP) that compromises the ability to achieve the primary study objectives or compromises subject safety.

If any of the above occurs, the study will be terminated if careful review of the overall risk/benefit analysis described in Section 6.4 demonstrates that the assumptions have changed and that the overall balance is no longer acceptable. In these circumstances termination can only take place with the agreement of the investigator and sponsor. The MHRA and EC will be informed of study termination.

If it becomes necessary to consider termination of the study after dosing has begun, dosing may be suspended pending discussion between the investigator and sponsor. Dosing will be stopped immediately on safety grounds.

The study may be terminated early or suspended at the request of the MHRA or EC.

8.7 Lost to Follow-up

A subject will be considered lost to follow-up if they fail to return for scheduled visits and cannot be contacted by the clinical unit.

If a subject fails to return to the clinical unit for a required study visit:

- The clinical unit must attempt to contact the subject and reschedule the missed visit as soon as possible
- Before a subject is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the participant (eg 3 telephone calls on 3 separate occasions and, if necessary, an email or letter to the participant's last known email/postal address). These contact attempts should be documented in the subject's source
- If the subject cannot be contacted, they will be considered lost to follow-up

8.8 Randomisation

This is a double-blind, randomised, single centre, parallel-group, single-dose, dose-escalation, placebo-controlled study; therefore, a randomisation schedule will be produced.

The original randomisation schedule and proof of quality control (QC) procedures will be held by the Data Sciences department at Quotient Sciences until the study is archived, at which time the randomisation materials will be retained in the ISF.

Subjects will be randomised immediately before dosing.

8.8.1 Subject Numbers

Subject numbers will be allocated on the morning of dosing according to the code 001 to 064 using the lowest number available. Replacement subjects will be allocated subject numbers 901 to 964, where the last 2 digits are the same as those of the original subject (eg if Subject 005 withdraws, the replacement will have Subject Number 905 and will receive the same regimen as Subject 005).

Subject numbering by cohort is provided in Table 3.

Table 3 Subject Numbers by Cohort

Cohort	Subject Numbers
1	001 to 008
2	009 to 016
3	017 to 024
4	025 to 032
5	033 to 040
6	041 to 048
7	049 to 056
8	057 to 064

Each cohort will be split into 2 groups (sentinel and main), see Section 8.1 for details.

8.8.2 Treatment Allocation

Using a computer-generated randomisation schedule, subject numbers will be allocated to treatment in a 3:1 ratio. The allocation for the sentinel group of each cohort will be balanced with one subject receiving active IMP and one receiving placebo. The allocation for the main group of each cohort will be unbalanced with 5 subjects receiving active IMP and 1 subject receiving placebo.

A treatment allocation list will be produced prior to dosing using the randomisation schedule and will be retained in the ISF.

8.8.3 Blinding

This is a double-blind study. Treatment assignment will not be known to the subjects, the sponsor or the staff who are involved in the clinical evaluation of the subjects and the analysis of data. The randomisation schedule and disclosure envelopes will be generated by an unblinded statistician at Quotient Sciences according to Quotient Sciences SOPs. The unblinded statistician will not be involved in any decisions relating to populations for analysis prior to unblinding.

Prior to database lock and unblinding, all original randomisation materials, including the original final signed and dated randomisation schedule, will be held by the Quality Assurance (QA) department at Quotient Sciences. The Data Sciences department will not have access to the randomisation schedule before database lock and unblinding.

Interim PK parameter estimations will be performed using bioanalytical data applied with subject aliases in order to maintain the study blind.

There may be instances where interim PK data have the potential to be treatment revealing eg missed blood sampling occasions. In these cases, every effort will be made by the pharmacokineticist to maintain the study blind by appropriate presentation of data to the study team. Data demonstrating extremes of exposure will always be presented, regardless of the potential to reveal the study blind.

The unblinded Qualified Person (QP) or designee at the clinical site will receive a copy of the final randomisation schedule for preparation of the study drug and preparation of the treatment allocation list. A copy of the randomisation schedule will also be made available to the laboratory performing the bioanalysis to allow selective analysis of drug concentrations and to the pharmacovigilance provider for potential safety reporting.

Two sets of disclosure envelopes (ie sealed envelopes containing individual subject randomisation details) will be provided. One set will be held in the clinical area and the other retained in the ISF. These may be used in the event of an emergency by the investigator. Any request for information on the randomisation schedule after initial issue must be made using a randomisation disclosure form, except in the case of emergency unblinding, which must be recorded on the emergency unblinding form. Access to study drug assignment will be immediately available if the investigator deems it necessary to break the study blind in the interest of a subject's medical safety, in case of a medical emergency, or if warranted during scheduled safety reviews. The sponsor medical responsible and the sponsor project manager must be contacted within 24 h following disclosure of study drug assignment.

Details of any disclosure of the randomisation schedule will be documented and retained in the ISF. The sponsor will be notified if the study blind is broken.

The study blind will be broken after the study database has been locked and the safety population has been defined. Any subsequent request for issue of the randomisation schedule prior to unblinding must be made using a randomisation disclosure form.

9 Selection of Subjects

Quotient Sciences must have a full medical history from each subject's general practitioner (GP) within the last 12 months, prior to enrolment for the study.

Subjects will be recruited from the Quotient Sciences panel or by direct advertising to the public.

Before subjects are admitted to the clinical unit, The Over Volunteering Prevention System (TOPS) will be checked to ensure that each subject has not participated in a study at another site within at least 3 months of the dosing date.

9.1 Informed Consent

Subjects will be provided with a written explanation of the study at least the day before the screening visit. A physician or nurse will explain to each subject the nature of the study, its purpose, expected duration, the benefits and risks and any study lifestyle restrictions involved in study participation. Subjects will be informed that, for safety reasons, brief details of their involvement in the study may be revealed to other units and companies that carry out clinical studies in the local area. Subjects will then be given the opportunity to ask questions and will be informed of their right to withdraw from the study without prejudice. After this explanation and before entering the study, the subject will voluntarily sign an informed consent form (ICF). Until written consent has been obtained from the subject no study specific procedure or investigation will be performed. If an amendment is made to the participant information sheet (PIS), participants will be re-consented to the most current version of the ICF(s) where appropriate.

9.2 Inclusion Criteria

1. Healthy males (Cohorts 1 to 7) or healthy WONCBP (Cohort 8)
2. 18 to 55 years (Cohorts 1 to 7) or 18 to 60 years (Cohort 8) of age at the time of signing informed consent
3. Body mass index (BMI) of 18.0 to 30.1 kg/m^2 as measured at screening
4. General good physical health determined by medical and surgical history, physical examination, 12-lead ECG, vital signs and clinical laboratory tests

5. 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
6. A resting HR between ≥40 and ≤90 bpm measured after 10 min rest in supine position at screening, admission and pre-dose
7. ECG recording without clinically significant abnormality, including QTcF measure of ≤450 msec (male) or ≤470 msec (female) at screening, admission and pre-dose
8. Having had no febrile seizures or infectious illness for at least 7 days prior to administration of the IMP (Day 1)
9. Must be willing and able to communicate and participate in the whole study
10. Must provide written informed consent
11. Must agree to adhere to the contraception requirements defined in Section 9.4 and the life-style restrictions described in Section 9.6

Inclusion criteria 4, 5, 6, 7 and 8 from the list above will be re-assessed at admission/pre-dose.

9.3 Exclusion Criteria

1. Subjects who have received any IMP in a clinical research study within the 3 months or 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 alcohol breath test at screening or admission
7. Current smokers and those who have smoked within the last 12 months. As confirmed by a breath carbon monoxide reading of greater than 10 ppm at screening or admission
8. Current users of e-cigarettes and nicotine replacement products and those who have used these products within the last 12 months
9. Females of childbearing potential including those who are pregnant or lactating (all female subjects must have a negative serum pregnancy test at screening and admission). A woman is considered of childbearing potential unless she is permanently sterile (hysterectomy, bilateral salpingectomy, bilateral tubal ligation, bilateral tubal occlusion and bilateral oophorectomy) or is postmenopausal (had no menses for 12 months without an alternative medical cause and a serum follicle stimulating hormone [FSH] concentration ≥40 IU/L)
10. Subjects who do not have suitable veins for multiple venepunctures/cannulation as assessed by the investigator or delegate at screening
11. Clinically significant abnormal biochemistry, haematology, coagulation or urinalysis (especially AST, ALT, gamma glutamyl transpeptidase [GGT], ALP, creatinine, and BUN) as judged by the investigator (laboratory parameters are listed in Appendix 1). Subjects with Gilbert's syndrome are allowed
12. Confirmed positive drugs of abuse test result (drugs of abuse tests are listed in Appendix 1)

13. Positive hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (HCV Ab) or human immunodeficiency virus (HIV) results
14. Evidence of renal impairment at screening or admission, as indicated by an estimated CLcr of <80 mL/min using the Cockcroft-Gault equation
15. History of clinically significant cardiovascular, renal, hepatic, neurological (especially seizures), immunological, psychiatric, myopathies, bleeding tendency, respiratory and particularly GI disease, especially peptic ulceration and chronic gastritis, GI bleeding, ulcerative colitis, Crohn's Disease or Irritable Bowel Syndrome, as judged by the investigator
16. History of additional risk factors for Torsades des Pointe (eg heart failure, hypokalaemia, family history of long QT syndrome)
17. Rare hereditary problems of fructose intolerance, glucose-galactose malabsorption or sucrose-isomaltase insufficiency
18. Any relevant GI complaints within 7 days of dosing
19. Subjects with a history of cholecystectomy or gall stones (Cohort 7 only)
20. 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)
21. 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
22. Donation or loss of greater than 500 mL of blood within the previous 3 months or more than 100 mL within 30 days before signing ICF to this trial
23. Subjects who are taking, or have taken, any prescribed or over-the-counter drug (including anti-acid drugs) or vitamins/herbal remedies (eg St. John's Wort and others which are known to interfere with the CYP3A4 and P-gp metabolic pathways) or HRT in the 21 days before IMP administration. Administration of up to 4 g of paracetamol per day within 7 days of IMP administration is allowed (See Section 11.4)
24. Surgery within 12 weeks prior to screening, with the exception of appendectomy
25. Any surgery (eg gastric bypass) or medical condition that may affect absorption of orally administered drugs
26. Failure to satisfy the investigator of fitness to participate for any other reason

Exclusion criteria 6, 7, 9, 10, 11, 12, 14, 18, 23 and 26 the list above will be re-assessed at admission/pre-dose.

Healthy subjects who do not meet the inclusion/exclusion criteria for a study should not be enrolled into the study without exception.

9.4 Contraception

Male Subjects

Male subjects who are sexually active must use, with their partner of child-bearing potential, a condom plus an approved method of effective contraception from the time of informed consent to 90 days after study discharge. The following methods are acceptable:

- Partner's use of combined (oestrogen and progestogen-containing) hormonal contraception associated with inhibition of ovulation:
    - oral
    - intravaginal
    - transdermal

- Partner's use of progestogen-only hormonal contraception:
  - oral
  - injectable/implantable
  - intrauterine hormone-releasing system

- Partner's use of implantable intrauterine device

- Surgical sterilisation (for example, vasectomy or partner's bilateral tubal occlusion)

Alternatively, true abstinence is acceptable when it is in line with the subject's preferred and usual lifestyle. If a subject is usually not sexually active but becomes active, they, with their partner, must comply with the contraceptive requirements detailed above.

Women of Non-Child Bearing Potential

Female subjects who are not of childbearing potential do not need to use any methods of contraception. A woman is considered of childbearing potential (WOCBP) unless post-menopausal or permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy, bilateral tubal ligation, bilateral tubal occlusion and bilateral oophorectomy. A post-menopausal state is defined as no menses for 12 months without an alternative medical cause and confirmed by a FSH result of ≥40 IU/L.

These contraception requirements are aligned with guidance issued by the Heads of Medicines Agency: Clinical Trials Facilitation Group [9].

9.4.1 Exposure to Partners During the Study

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. Therefore, a condom should be used by all male subjects throughout the study and for 1 week (relative to systemic exposure based on conservative estimate of 5 half-lives of the IMP) after study discharge.

9.4.2 Sperm Donation

Subjects should not donate sperm for the duration of the study and for at least 90 days after study discharge.

9.5 Pregnancy in a Trial Subject's Partner or in a Trial Subject

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 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, ie, within 24 h, with the same procedure and timelines as for SAEs (see Section 16.3.1). 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 (ie, 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.

9.6 Additional Study Restrictions

The following additional restrictions will be in place for the duration of the study:

1. Subjects must abstain from alcohol during the 24 h prior to screening and the 48 h prior to admission until discharge from the study.
2. Subjects must not drink liquids or eat food containing grapefruit or cranberry from 72 h prior to admission until discharge from the study.
3. Subjects must not drink liquids or eat food containing caffeine or other xanthines from 24 h prior to admission until discharge from the study.
4. Subjects should refrain from eating food containing poppy seeds for 48 h prior to screening and for 48 h prior to admission until discharge from the study.
5. Subjects must not take part in any unaccustomed strenuous exercise from the 72 h before the screening visit and then from 72 h prior to admission until discharge from the study.
6. Subjects must agree to not donate blood or blood products for 90 days after discharge from the study.

10 Study Procedures

Study procedures will be performed as detailed in the study schedule of assessments in Appendix 2, and in accordance with Quotient Sciences SOPs unless otherwise stated in this protocol.

10.1 Screening

Within the 28 days preceding first dose, all subjects will be required to undergo a screening visit. Screening procedures will be carried out in accordance with the study flow chart in Appendix 2.

If the start of the study is delayed for any reason so that the interval between screening and first dose exceeds 28 days, all or part of the screening procedures may be repeated at the discretion of the investigator.

Subjects previously screened generically may participate in this study provided they meet the subject selection criteria. Procedures required by this protocol will only be done if they were not performed during generic screening. All screening data must be obtained within 28 days prior to administration of study medication, as stipulated above.

Screening safety procedures such as safety bloods, ECGs, vital signs and urinalysis can be repeated as clinically indicated under the discretion of the investigator or sub-investigator if there is a concern regarding a subject's safety or eligibility to participate in the trial.

10.1.1 Subject Re-Screening

This study permits the re-screening of a subject who has discontinued the study as a pre-treatment failure (ie subject has not been randomised); the reason for failure must be temporary and expected to resolve. If re-screened, the subject must be re-consented.

10.2 Admission and Pre-dose Procedures

The identity of the subjects will be confirmed at admission and pre-dose.

In addition, the ongoing eligibility of subjects will be re-assessed at admission/pre-dose, as described in Sections 9.2 and 9.3.

Admission safety procedures such as safety bloods, ECGs, vital signs, urinalysis and drugs of abuse tests can be repeated as clinically indicated under the discretion of investigator or sub-investigator if there is a concern regarding a subject's safety or eligibility to participate in the clinical trial.

Reserve subjects for the first dose occasion, in any group, will not require admission procedures to be repeated, if dosing is within 2 days.

The subjects will be admitted to the clinical unit on the morning before dosing (Day -1).

The admission and pre-dose procedures are presented in Appendix 2.

10.3 Study Day Procedures

10.3.1 Blood Volume

The number and timing of samples may be amended following any interim PK parameter estimations. However, in this case, the total blood volume for each subject will not exceed 550 mL in a 4 week period.

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

10.3.2 Timing of Procedures

There are times where the protocol requires more than one procedure to be completed at the same time point. In these instances the following will apply to post-dose time points:

PK samples should take priority over other procedures scheduled at the same time point.

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

ECGs 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 and no other procedures will be performed within this time window. Where required, procedures may be performed in the rest period prior to extractions. Other assessments, eg physical examinations etc, will be performed within the required time windows.

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

10.3.3 Discharge from the Clinical Unit

A subject will be allowed to leave the premises without additional Investigator or delegate review, following completion of study-specific procedures at 72 h post-dose providing that:

- no AEs have been reported during the study visit
- the subject responds in the affirmative when asked if they are feeling well

If any of these conditions are not met, then the subject will only be allowed to leave the clinical unit with the authorisation of the investigator or appropriately qualified delegate.

There will be no continued provision of the study intervention or treatment for subjects as this study involves healthy volunteers only.

10.3.4 Medical Supervision

A physician will be responsible for the clinical aspects of the study and will be available at all times during the study. A physician must be present in the clinical unit until 24 h post-dose for sentinel and main groups of each cohort and there-after on an on-call basis. In accordance with the current Association of the British Pharmaceutical Industry guidelines [10], each subject will receive a card stating the telephone number of the investigator and the 24/7 contact details of the Quotient on-call medic.

10.3.5 Follow-up

Follow-up procedures will be carried out according to the schedule presented in Appendix 2.

11 Dosing of Subjects

11.1 Food and Fluid Intake

The calorie/fat content of meals is not required to be controlled during this study, with the exception of a standard high fat breakfast for Cohort 7.

Subjects will be allowed water up to 1 h before the scheduled dosing time and will be provided with 240 mL of water at 1 h post-dose. Water will be allowed ad libitum after 1 h post-dose. Decaffeinated fluids will be allowed ad libitum from lunch time on the day of dosing.

For Cohorts 6 to 8: Fluid intake will be recorded in the subjects' source whilst the subject is resident in the clinical unit.

If, for technical reasons, dosing is delayed for more than 2 h beyond the expected dosing time, subjects will receive 200 mL of Lucozade Sport at the originally scheduled dosing time, or earlier if possible.

Fasted Dosing

Subjects will be provided with a light snack and then fast from all food and drink (except water) for a minimum of 10 h on the day prior to dosing (Day -1) until approximately 4 h post-dose, at which time lunch will be provided. An evening meal will be provided at approximately 10 h post-dose and an evening snack at approximately 14 h post-dose. On subsequent days, meals will be provided at appropriate times.

Fed Dosing (Cohort 7 only)

Subjects will be provided with a light snack and will fast (for a minimum of 10 h) from all food and drink (except water) until the following morning, when they will be provided with a standard high fat breakfast.

The breakfast should be consumed over a maximum period of 25 min, with dosing occurring 30 min after the start of breakfast. Subjects should be encouraged to eat their meal evenly over the 25 min period. It is acknowledged that some subjects will take less time to eat, but dosing should still occur 30 min after the start of breakfast.Subjects must consume at least 90% of the pre-dose breakfast in order to be eligible for dosing.

The acceptable deviation for the pre-dose meal from the nominal time point is:

- Pre-dose meal will be provided within ± 5 min of the nominal time point

Lunch will be provided at approximately 4 h post-dose, an evening meal at approximately 10 h post-dose and an evening snack at approximately 14 h post-dose. On subsequent days, meals will be provided at appropriate times.

11.2 Administration of Test Preparations

Specific details of IMP(s) and doses to be administered are provided in Section 5.2 and Section 8.1, respectively. Subjects will be dosed on the morning of Day 1.

The exact time of dosing will be decided based on logistics and will be documented in the source. The order in which regimens are dosed may be subject to change due to logistical reasons. Details of the procedures adopted for maintaining the study blind during dosing are provided in Section 8.8.3.

It is planned that each ascending dose will be administered in a staggered 'sentinel' dose design. All cohorts will be split into 2 groups; in Cohort Xa, 1 subject will receive active IMP and 1 subject will receive placebo; in Cohort Xb, 5 subjects will receive active IMP, and 1 subject will receive placebo, where X indicates the number of the cohort.

The interval between follow-up visits, may be changed, if data collected during the study support the change.

Subjects will receive a total of one administration of DNDI-0690 capsule or matching placebo on one occasion.

240 mL of non-carbonated water will be given immediately following oral administration. Additional water may be given with the IMP if required and will be recorded in the source as appropriate.

11.3 Dosing Compliance

During all clinical phases of the study, subjects will be observed by study staff to assure compliance to all study procedures, including dose administration.

Mouth and hand checks will be conducted after dosing to ensure the capsule(s) has been swallowed.

The date and time that each subject is dosed will be recorded in the subject's source data. Any violation of compliance will require evaluation by the investigator and sponsor to determine if the subject can continue in the study.

11.4 Prior and Concomitant Medications

No prescribed or over-the-counter drug (including anti-acid drugs) or vitamins/herbal remedies (eg St. John's Wort and others which are known to interfere with the CYP3A4 and P-gp metabolic pathways) or HRT will be permitted from 21 days before IMP administration until the follow-up visit except up to 4 g of paracetamol in 24 h and those deemed necessary by the investigator to treat AEs (see also Section 9.3). Any medications used will be recorded in the source.

Emergency equipment and drugs will be available within the clinical unit as per current standard procedures. In the unlikely event that they are required, their use will be documented.

12 Assessment of Efficacy

Not applicable for this Phase I study.

13 Assessment of Pharmacokinetics and Pharmacodynamics

13.1 Assessment of Pharmacokinetics

13.1.1 Pharmacokinetic Blood Sampling

Venous blood samples will be collected from the subjects by a trained member of the clinical team. Consent will be collected from the subjects for use of these samples for the purposes of the proposed study. Pharmacokinetic analysis will be carried out on blood samples.

Blood samples are sent for laboratory testing in linked anonymised form (subject number and initials only). This information is able to be linked directly to the volunteer by the Quotient research team and study monitor, however not by the laboratory staff or Sponsor.

Venous blood samples will be withdrawn via an indwelling cannula or by venepuncture according to the time schedule presented in Appendix 2.

The acceptable deviations from the nominal blood sampling times are as follows:

- The pre-dose samples will be taken ≤1 h before dosing

- 0 to 2 h post-dose samples will be taken within ± 2 min of the nominal post-dose sampling time
- 2.5 to 4 h post-dose samples will be taken within ± 3 min of the nominal post-dose sampling time
- 6 to 12 h post-dose samples will be taken within ± 5 min of the nominal post-dose sampling time
- 24 to 72 h post-dose samples will be taken within ± 30 min of the nominal post-dose sampling time

The timing and number of the samples may be amended following any interim PK parameter estimations, including collection over a longer duration. Any changes to blood sampling time points would be documented in the interim dose decision report and retained in the ISF.

Samples will be collected into appropriate tubes and shipped as specified by the bioanalytical laboratory. Details of sample tubes and processing will be contained in the Clinical Sample Processing Manual.

13.1.2 Pharmacokinetic Urine Sampling

Urine samples will be collected according to the time schedule presented in Appendix 2.

A pre-dose (blank) urine sample will be taken at least 1 h before dosing. All individual urine voids will be collected and pooled per collection period. The pooled sample will be weighed, the volume will be calculated and an aliquot of urine for each collection period will be shipped to the bioanalytical laboratory for analysis, according to Quotient Sciences SOPs, unless indicated otherwise by the sponsor.

Samples will be collected into appropriate containers containing bovine serum albumin and shipped as specified by the bioanalytical laboratory. Details of sample containers and processing will be contained in the Clinical Sample Processing Manual.

13.2 Assessment of Pharmacodynamics

Not applicable for this Phase I study.

13.3 Exploratory Assessments

13.3.1 Holter extracted ECGs

Continuous Holter monitoring will commence at least 1 h prior to dosing until 24 h post-dose, ie, after the blood sample for PK has been withdrawn at 24 h post-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. On Day 1, three pre-dose triplicates will be extracted and averaged to establish a baseline value.

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 MAD trial, eg, the Holter extracted ECGs will be analysed together with the Holter extracted ECGs collected in the MAD trial, and reported in a separate report.

Holter extraction window times will be recorded in the subject's source. The Holter data collected in this trial will not be part of the trial's clinical data base.

Where any scheduled extractions are missed a protocol deviation will be recorded, loss of leads or interruption of the recording outside of the scheduled extractions will not be considered a protocol deviation.

See Section 10.3.2 for details on timing of Holter extractions in relation to other procedures.

In case the extracted ECG data are analysed, the following analyses are planned and will be reported in a separate report:

Changes from baseline (average of the three pre-dose extracted ECG triplicates) with the extracted ECG triplicates recorded on Day 1 will be calculated for each parameter:

- Categorical analysis for RR, HR, PR, QRS, QT, QTcF, QTcB, ΔHR, ΔRR, ΔPR, ΔQRS, ΔQT, ΔQTcF and ΔQTcB according to the thresholds defined below.
- ECG morphological analysis.

The pre-defined thresholds for the categorical analysis for maximum actual values will be:

- QTc interval > 450 msec (male) / > 470 msec (female)
- QTc interval > 480 msec
- QT/QTc interval > 500 msec

and for maximum changes from baseline:

- ΔQTc interval > 30 msec
- ΔQTc interval > 60 msec

All Holter extracted ECGs parameters and their changes from baseline will be summarised by dose regimen and time-point.

13.3.2 Urine Sampling for Drug-related Renal Injury Exploratory Biomarkers

Urine samples for drug-related renal injury exploratory biomarkers will be collected according to the time schedule presented in Appendix 2.

Samples will be taken as an aliquot from PK urine collection after the sample has been weighed. Urine samples will be collected and processed as detailed in the Clinical Sample Processing Manual.

Samples will be stored and may be analysed at the end of the trial retrospectively or as required during the study if medically relevant changes in the renal parameters of safety laboratory assessments are observed (ie, BUN, creatinine):

- α-GST
- CLU
- CysC
- KIM-1
- NGAL

- OPN
- ALB
- TPRO

The samples will be stored until finalisation of the clinical study report, and destroyed if no analyses are to be performed.

14 Assessment of Safety

14.1 Safety Definitions

14.1.1 Adverse Events

An AE is any untoward medical occurrence in a clinical trial subject where a medicinal product has been administered, which does not necessarily have a causal relationship with this treatment. For this study, the untoward medical occurrences that may occur in a subject before receiving study medication are referred to as a “pre-dose AE” or once a medicinal product has been administered, as an TEAE and should be collected within the database.

It can therefore be any unfavourable and unintended sign (eg 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 (ie. ECG …) or laboratory results which are assessed as “clinically significant”.

14.1.2 Clinically Significant Laboratory Procedures/Abnormalities

For every laboratory assessment, the investigator will evaluate if the laboratory test 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 fact 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.

Laboratory/procedures (ie. ECG...) abnormalities should 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 (eg, acute pancreatitis instead of each finding separately: high levels of amylase, high levels of lipase, abdominal pain and vomiting; eg “hypokalaemia” rather than “decreased potassium levels”).

14.1.3 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).

14.1.4 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: ie. 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: ie. the AE resulted in a substantial disruption of the subject’s ability to conduct normal activities;
- consists of a congenital anomaly or birth defect: ie. 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: ie, 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 serious adverse event/reaction.

14.1.5 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 ie. the IB.

14.1.6 Suspected Unexpected Serious Adverse Reactions (SUSARs)

SUSARs are SAEs which are believed to be related to an IMP and are both unexpected (ie 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 (see Section 16.3.2 for details on reporting SUSARs).

14.2 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.

14.3 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.

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.

14.4 Analysis of Events

14.4.1 By the Investigator

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

- For severity
- For causality
- As serious or non-serious

14.4.2 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, each change in severity will be recorded in the source documents until the event resolves. However, only one AE and the maximum severity will be recorded in the source 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.

14.5 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 (ie. 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 (ie. 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 adverse event and administration of study agent are related in time, and a direct association can be demonstrated.
- **Probably related**. The adverse event and administration of study agent are reasonably related in time, and the adverse event is more likely explained by study agent than other causes.
- **Possibly related**. The adverse event and administration of study agent are reasonably related in time, and the adverse event can be explained equally well by causes other than study agent.
- **Probably not related**. A potential relationship between study agent and the adverse event could exist (ie. the possibility cannot be excluded), but the adverse event is most likely explained by causes other than the study agent.
- **Not related.** The adverse event 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".

14.5.1.1 Adverse Event Seriousness Assessment

The Investigator will evaluate the seriousness of any event.

14.5.2 By the Sponsor

The Investigator is responsible for assessing seriousness and relatedness of an AE. In parallel, 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 for each AE. The Investigator's decision will be independent of the Sponsor's.

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.

14.6 Laboratory Measurements

Venous blood and urine samples will be collected from the subjects by a trained member of the clinical team. Consent will be collected from the subjects for use of these samples for the purposes of the proposed study.

Blood and urine samples are sent for laboratory testing in linked anonymised form (subject number, initials, and the subjects' gender and date of birth for analytical reasons). This information is able to be linked directly to the volunteer by the Quotient research team and study monitor, however not by the laboratory staff or sponsor.

Safety laboratory tests and virology will be carried out on blood samples, and drugs of abuse tests and urinalysis will be carried out on urine samples. The research will not involve analysis or use of human DNA.

Blood and urine samples results will be reviewed by a physician and acted upon before the subject is dosed or receives their next dose, or is released from the study, as is appropriate. A list of the laboratory parameters measured is presented in Appendix 1.

14.6.1 Haematology, Clinical Chemistry and Coagulation

Laboratory tests will be performed by The Doctors Laboratory according to the time schedule presented in Appendix 2. Blood samples will be collected and processed as detailed in the Clinical Sample Processing Manual. Scheduled blood samples will be taken following an 8 h fast.

The acceptable deviations from the nominal blood sampling time points for laboratory assessments are:

- The pre-dose blood sample will be taken ≤2 h before dosing
- Post-dose blood samples will be taken ± 30 min from the nominal blood sampling time except when the time point coincides with the PK blood sampling time. In this situation, the time window for the PK sample applies.

CLcr will be calculated at screening for eligibility purposes and at the time points indicated in Appendix 2 by The Doctors Laboratory using the Cockcroft-Gault equation and body weight:

$$\text{CLcr (mL/min)} = \frac{(140\text{-age [years]}) \times (\text{body weight [kg]}) \ (\times 1.23)}{\text{serum creatinine (µmol/L)}}$$

14.6.2 Urinalysis

Urinalysis will be performed on-site using a dipstick according to the time schedule presented in Appendix 2. Urine samples will be collected and processed as detailed in the Clinical Sample Processing Manual. Any urine used for urinalysis will be added back to the full sample for PK analysis. If microscopy is required, a urine sample (approximately 20 mL) will be taken from the urine PK collection, after the sample has been weighed and sent to The Doctors Laboratory.

The acceptable deviations from the nominal urine sampling time points for urinalysis are:

- Post-dose urine samples will be taken ± 2 h from the nominal urine sampling time

14.6.3 Pregnancy Test

Serum and urine pregnancy tests will be performed as detailed in Appendix 2. The samples will be collected and processed as detailed in the Clinical Sample Processing Manual.

14.6.4 Follicle-Stimulating Hormone Test (for Post-menopausal Females Only)

Serum FSH tests will be performed as detailed in Appendix 2. The samples will be collected and processed as detailed in the Clinical Sample Processing Manual.

14.6.5 Drug Screen

A urine drug screen will be performed on-site using a dipstick method according to the time schedule presented in Appendix 2. The sample will be collected and processed as detailed in the Clinical Sample Processing Manual. Subjects will be screened for the drugs of abuse listed in Appendix 1.

14.6.6 Alcohol Breath Test

An alcohol breath test will be performed according to the time schedule presented in Appendix 2. A positive result will exclude the subject from dosing during that admission.

14.6.7 Carbon Monoxide Breath Test

A carbon monoxide breath test will be performed according to the time schedule presented in Appendix 2. A result of greater than 10 ppm will exclude the subject from dosing during that admission.

14.6.8 Troponin I

Serum samples for Troponin I will be collected at the time points indicated in Appendix 2. Time points for sample collection may be altered based on emerging data, see Section 8.2.1.

14.6.9 Abnormal Laboratory Findings

In cases where laboratory findings are outside the normal range and the investigator believes that the results may be of clinical significance, repeat sampling may be requested as clinically indicated. If the abnormal finding is clinically significant, appropriate actions will be taken eg, the subject will not be entered into the study or the subject may be withdrawn from the study drug. The subject will be referred to their GP or other appropriate provider for further care. The same will apply if the results of the HBsAg, HCV Ab or HIV test are positive and in addition the investigator will ensure that adequate counselling is available if requested.

Abnormal results at follow-up assessments will also require repeat testing if the investigator believes the results may be of clinical significance.

Any clinically significant abnormality, including changes from baseline, must be reported as an AE.

Additional blood and/or urine samples may be taken for safety tests. Furthermore, additional assays outside those specified in the protocol may be performed for safety reasons as requested by the investigator.

14.7 Vital Signs Measurements

Blood pressure and HR will be measured by an automated recorder after the subject has been in a supine position for a minimum of 10 min according to the time schedule presented in Appendix 2. Where possible the same arm will be used for all blood pressure measurements. Should the designated arm need to be changed due to, for example, re-cannulation, this will not be classed as a protocol deviation. Tympanic body temperature will be measured at screening and pre-dose only. The acceptable deviations from the nominal vital signs measurement time points are:

- The pre-dose vital signs measurements will be taken ≤2 h before dosing.
- Post-dose vital signs measurements will be taken ± 15 min from the nominal post-dose time points.
- Discharge vital signs measurements will be taken ± 1 h from the nominal time point.

If a subject shows an abnormal assessment at any stage, repeat measurements may be made and the abnormality followed to resolution if required. Additional measurements may be taken as deemed necessary by the investigator.

Any clinically significant abnormality, including changes from baseline, must be reported as an AE.

14.8 ECG Measurements

12-lead ECGs will be measured after the subject has been in the supine position for a minimum of 10 min according to the time schedule presented in Appendix 2. ECGs will be taken in triplicate at pre-dose (for baseline), all post-dose measurements will be taken as a single ECG. The acceptable deviations from the nominal ECG measurement time points are:

- The pre-dose ECG measurements will be taken ≤2 h before dosing
- Post-dose ECG measurements will be taken ± 15 min from the nominal post-dose time point.

- Discharge ECG measurements will be taken ± 1 h from the nominal time point.

If a subject shows an abnormal assessment at any stage, repeat measurements may be made and the abnormality followed to resolution if required. Additional measurements may be taken as deemed necessary by the investigator or sub-investigator. The PI or sub-investigator must double-check the results.

All safety ECG (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 Quotient at the end of the study.

Any clinically significant abnormality, including changes from baseline, will be reported as an AE.

14.9 Body Weight and Height

The subject's body weight and height will be measured as detailed in Appendix 2.

14.10 Physical Examination

Subjects will undergo a physical examination as detailed in Appendix 2.

14.11 Additional Safety Procedures

Additional non-invasive procedures that are already specified in the protocol may be performed, if it is believed that an important effect of the IMP(s) is occurring or may occur at a time when no measurements are scheduled, or if extra procedures are needed in the interests of safety.

Additional blood samples for safety assessments may be taken if required by the investigator at any point.

15 Statistics and Data Analysis

15.1 Sample Size Justification

The study is exploratory and no formal sample size calculation has been made. Based on experience from previous studies of a similar design, a total of 8 subjects are to be enrolled per cohort and a minimum of 6 evaluable subjects per cohort are considered sufficient.

15.2 Data Management

Data management will be performed by Quotient Sciences.

All study data recorded in the source (refer to Source Document Identification List for details), will be recorded in a validated study database that has an audit trail to log all subsequent changes to the data. All queries will be resolved within the study database.

AEs, medical history and medications will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) (v22.0 or the more recent version at the time of study initiation) and the World Health Organisation (WHO) Drug Dictionary Global Drug Reference (2019 or the most recent version at the time of study initiation), respectively. An independent coding review will also be performed within the Data Sciences department.

Clinical chemistry, haematology and coagulation data (and other safety laboratory data) will be collected by a central laboratory (The Doctors Laboratory) and stored electronically in their clinical pathology system. The data will be transferred electronically to Quotient Sciences and all demographic details and sample dates will be cross-referenced with the corresponding data on the study database. All queries will be resolved with the assistance of laboratory staff, or if necessary, clinical staff.

The database will be closed after all queries have been resolved, including any reconciliation with external databases deemed necessary. The database will be locked when all criteria listed in the Data Management Plan (DMP) are met.

Further details are addressed in the DMP.

15.3 Pharmacokinetic Data Analysis

The plasma concentration data for DNDI-0690 provided by SGS Belgium will be analysed by Quotient Sciences, using Phoenix WinNonlin v8.0 or a more recent version (Certara USA, Inc., USA).

Plasma concentration data will be tabulated and plotted for each subject for whom DNDI-0690 concentrations are quantifiable. PK analysis of the concentration time data obtained will be performed using appropriate non-compartmental techniques to obtain estimates of the following PK parameters, where possible and applicable:

- **T_{max}:** the elapsed time from dosing at which C_{max} was apparent
- **C_{max}:** the maximum observed concentration
- **$C_{max/D}$:** the maximum observed concentration normalised for dose
- **$C_{max,\ norm}$:** the maximum observed concentration normalised for dose and body weight
- **$AUC_{(0–24)}$:** area under the curve from 0 time to 24h
- **$AUC_{(0–t)}$:** area under the curve from 0 time to last measurable concentration
- **$AUC_{(0–t),\ norm}$:** area under the curve from 0 time to last measurable concentration normalised for dose and body weight
- **$AUC_{(0–inf)}$:** area under the curve from 0 time extrapolated to infinity
- **$AUC_{(0–inf)/D}$:** area under the curve from 0 time extrapolated to infinity normalised for dose
- **$AUC_{(0–inf),\ norm}$:** area under the curve from 0 time extrapolated to infinity normalised for dose and body weight
- **$AUC_{t\text{-}inf}$:** percentage of $AUC_{(0–inf)}$ extrapolated beyond last measured time point
- **Points terminal:** the number of points used to determine the elimination half-life
- **$T_{1/2}$:** the apparent elimination half-life
- **$C_{l/F}$:** apparent plasma clearance after oral administration
- **$V_{z/F}$:** apparent volume of distribution based on area for oral dose
- **MRT:** mean residence time
- **lambda-z:** the slope of the apparent elimination phase

The urine concentration data for DNDI0690 will be analysed for the following PK parameters:

- **Ae:** amount excreted
- **Fe:** fraction of dose excreted
- **CLr:** renal clearance

Amount excreted (Ae) and fraction excreted (Fe) will be calculated for 0-24 and 0-72 h.

Interim PK parameter estimations will be provided for dose escalation, as described in Section 8.2.

Further details of the PK data analysis will be included in the Reporting and Analysis Plan (RAP).

15.4 Statistical Data Analysis

Production of summary tables, figures and listings for this study will be performed by Quotient Sciences using the statistical package SAS (v9.4 or more recent version).

In general terms, categorical data (including TEAEs) will be presented using counts and percentages, while continuous variables will be presented using the mean, median, standard deviation, minimum and maximum. Additional statistics will be provided for PK-related data including coefficient of variation (CV%), geometric mean, geometric CV% and geometric n (ie the number of subjects with an observation that were included in the natural logarithmic transformation).

Descriptive summaries of all baseline characteristics and safety data (AEs, vital signs, ECGs and safety laboratory assessments) by all placebo dose subjects combined, all DNDI-0690 dosed subjects combined and for each dose level of DNDI-0690 will be provided (including changes from baseline as required).

Descriptive summaries for all PK data by dose level of DNDI-0690 will be provided.

In addition, dose proportionality will be assessed across dose groups (assuming same prandial status and same gender) using the power model. The power model will be applied separately to the ln(AUC) and ln(Cmax) values. A point estimate and its 90% confidence interval will be calculated for the population mean slope together with the acceptance range. Dose proportionality will be considered as statistically proven when the 90% confidence interval for the slope in completely contained in the acceptance range (1+ln(0.5)/ln(r)); 1+ln(2)/ln(r)) where r is the ratio highest/lowest dose. “ln” denotes the natural logarithm.

Further statistical analyses of PK parameters $AUC_{(0\text{-}t)}$, $AUC_{(0\text{-}inf)}$ and C_{max} will be performed to examine the effect of food in males (comparing fed and fasted cohorts at same dose level) and the effect of gender (comparing male and female cohorts at same dose level). Populations for analysis will be determined for safety (safety analysis set) and PK (PK analysis set) data after database lock using the criteria defined in the RAP.

The safety population will be defined after database lock but prior to study unblinding; all other populations will be defined after database lock and unblinding when the relevant data are available.

Further details relating to the statistical analysis will be included in the study-specific RAP including the following:

- Criteria to be used to define each of the analysis populations
- Additional detail covering the analyses and/or description of primary and secondary analyses and safety data
- Handling of missing data, unused or spurious data

- Handling of data from withdrawn subjects
- Unblinding procedures and maintaining the blind

Any deviations from the originally planned analysis as per the RAP will be documented in the clinical study report.

All safety (including safety ECGs parameters) and PK data will be listed.

All safety ECG parameters (see Section 13.3.1 for parameters) and their changes from baseline will be summarised by dose regimen and time point.

As described in Section 13.3, all Holter extracted ECGs and urinary biomarker data, will not be analysed as part of this study at this stage.

15.5 Interim Analysis

No formal interim analyses are planned for this study. After each cohort, safety and PK data will be reviewed before progressing to the next dose level, see Section 8.2.

16 Safety Reporting to Ethics Committees and Regulatory Authorities

16.1 Events Requiring Expedited Reporting

SUSARs (Section 14.1.6) are subject to expedited reporting to the MHRA, EMA and EC.

In addition to SUSARs, other safety issues may qualify for expedited reporting where they might materially alter the current benefit-risk assessment of an IMP or that would be sufficient to consider changes in the IMPs administration or in the overall conduct of the study, for instance:

- an increase in the rate of occurrence or a qualitative change of an expected serious adverse reaction, which is judged to be clinically important
- SAEs that occur after the subject has completed the clinical study where the sponsor considers them to be a SUSAR
- new events related to the conduct of the study or the development of the IMPs and likely to affect the safety of the subjects, such as:
    - an SAE which could be associated with the study procedures and which could modify the conduct of the study
    - a major safety finding from a newly completed animal study (such as carcinogenicity)
    - any anticipated end or temporary halt of a study for safety reasons and conducted with the same IMPs in another country by the same sponsor

16.2 Urgent Safety Measures

If Quotient Sciences or any of its staff or contractors becomes aware of an actual or potential urgent safety issue, then the sponsor must be immediately contacted so that appropriate urgent safety measures can be agreed. An urgent safety issue is defined as:

- An immediate hazard to the health or safety of subjects participating in a clinical study
- A serious risk to human health or potentially a serious risk to human health

An urgent safety issue may include issues with an investigational drug or comparators, study procedures, inter-current illness (including pandemic infections), concomitant medications, concurrent medical conditions or any other issues related to the safe conduct of the study or that pose a risk to study subjects.

In exceptional circumstances of imminent hazard and in order to safeguard the health or safety of individuals, Quotient Sciences may take urgent safety measures before informing the sponsor, but the sponsor must be informed immediately after implementation of the safety measure.

Quotient Sciences will take responsibility for informing appropriate competent authorities, and the EC.

16.3 Reporting

16.3.1 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 or serious adverse reaction. 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 for GCP.

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 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/SARs 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.

16.3.2 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. Quotient 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.

16.3.3 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 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

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.

16.3.4 Reporting of Urgent Safety Issues

Quotient Sciences is required to inform the appropriate competent authorities and the EC within 3 calendar days of the urgent safety issue.

16.4 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.

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.

17 Protocol Amendments and Deviations

17.1 Amendments

After the protocol has been submitted to the MHRA and/or EC, any amendment must be agreed by the investigator after discussion with the sponsor and will be formally documented.

All substantial amendments will be submitted to the MHRA and/or EC for an opinion as required by current regulations.

If the PIS and ICF are updated as a result of the substantial amendment, the new approved versions will be used to re-consent currently enrolled subjects and must be provided to additional subjects prior to their entry into the study.

17.2 Protocol Deviations

The study must be conducted in accordance with the Clinical Protocol and GCP. Should a protocol deviation occur, it must be promptly assessed in order to decide whether any of these non-compliances should be reported to the MHRA as a serious breach of GCP and the Clinical Protocol.

Protocol waivers are not acceptable.

Deviations from the protocol will be recorded in the source as noted by the clinical staff. This list will be accessible to the monitor at each monitoring visit. The sponsor will be informed of the minor deviations at minimum at the end of the corresponding cohort, and of the major ones as soon as detected.

Any protocol deviations assessed as major will be discussed with the sponsor in order to determine if the withdrawal criteria stated in Section 8.3 have been met.

18 Regulatory

18.1 Compliance

This study will be conducted in accordance with the protocol and with the following legislation:

- International Council for Harmonisation GCP Guidelines approved by the Committee for Medicinal Products for Human Use (CHMP) on 17 Jul 1996, which came into force on 17 Jan 1997, updated Jul 2002, Integrated Addendum E6 (R2) dated 09 Nov 2016 [11]
- The Medicines for Human Use (Clinical Trials) Regulations. Statutory Instruments 2004 No. 1031 [12]
- The Medicines for Human Use (Clinical Trials) Amendment Regulations. Statutory Instruments 2006 No. 1928 [13]
- The Medicines for Human Use (Clinical Trials) Amendment (No. 2) Regulations. Statutory Instruments 2006 No. 2984 [14]
- The Medicines for Human Use (Clinical Trials) Amendment Regulations. Statutory Instruments 2008 No. 941 [15]

In addition, the study will be performed according to the ethical principles outlined in the World Medical Association Declaration of Helsinki and its amendments [16].

The trial will be registered on a publically accessible database; clinicaltrials.gov.

18.2 Ethics Approval

Prior to the initiation of the study, the protocol and associated documentation must be given a favourable opinion by an EC. A copy of this written approval and any correspondence with the EC will be provided to the sponsor.

18.3 MHRA Approval

Prior to the initiation of the study, the Clinical Trial Authorisation application must be approved by the MHRA. A copy of this approval and any correspondence with the MHRA will be available at the clinical and sponsor sites. A copy of the MHRA approval will be provided to the EC.

18.4 Source Data

A study-specific source document identification list will be finalised with the sponsor prior to the start of the clinical phase of the study. The document will identify what data should be considered source data for this study.

For this study, electronic data capture will be used where possible and data will be automatically recorded into an electronic case report form. In instances where paper source documents are used, data to be transcribed into the electronic case report form will be identified using a Source Document Identification List, as governed by Quotient Sciences SOPs.

18.5 Declaration of the End of the Study

The end of the study is defined as the last visit of the last subject (eg follow-up assessment or phone call). Any changes to this definition will be notified as a substantial amendment (see Section 17.1).

The EC and MHRA should be notified in writing of the conclusion of the study within 90 days of the end of the study, or within 15 days if the study is terminated early, clearly explaining the reasons for the termination.

18.6 Document Storage and Archiving

All documentation and correspondence pertaining to the study (source data, raw data, letters etc) will be kept in accordance with the ICH guidelines for Good Clinical Practice 1996, updated 2002 Integrated Addendum E6 (R2) dated 09 Nov 2016 (ICH GCP Section 4.9.5) [11], The Medicines for Human Use (Clinical Trials) Regulations 2004 [12] and The Medicines for Human Use (Clinical Trials) Amendment Regulations 2006 [13],[14].

All study related documents will be retained for a minimum period of 25 years. After this time, the sponsor will be contacted to ascertain whether continued storage or destruction is required in accordance with current regulations.

18.7 Protection of Personal Data and Confidentiality

Personal data are securely stored to prevent unauthorised access, disclosure, dissemination, alteration or loss of information and unauthorised personal data processing. Access to personal information is restricted so that only personnel who are required to access personal data as part of their job role can do so. All personnel who access personal information are bound by a duty of confidentiality.

Technical arrangements surrounding the electronic storage and use of data are as follows:

- Computers storing electronic personal data are protected by antivirus software and the network on which computers are linked are protected by industry grade firewalls
- Off-site personnel can only access networked computers through a virtual private network
- Electronic access of data is limited according to user roles
- All data are stored on password protected computers

Organisational arrangements are as follows:

- All buildings are secured by key-card access
- Manual files of personal data are stored within locked cabinets that can only be accessed by authorised personnel
- Data security and/or confidentiality provisions are utilised in agreements with third parties
- Documented Back-up and disaster recovery procedures are in place
- Internal audit and compliance functions provide regulatory oversight

The personal data of volunteers will be pseudonymised in that they will only include health, initials, date of birth 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, Quotient personnel are contractually bound by a duty of confidentiality and receive training in this matter.

18.8 Data Security Breach

Quotient 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 QA personnel in accordance with Quotient Sciences SOPs. After robust assessment of data breaches, those deemed serious will be reported to the Sponsor and Information Commissioner's Office, as applicable.

19 Quality Control and Quality Assurance

QC of all data collected from this study will be performed in accordance with Quotient SOPs. This study (or elements thereof) may be subject to Quotient QA audit, in line with current internal auditing procedures. Similarly, the study (or elements thereof) may be subject to sponsor QA audit.

19.1 Monitoring

GCP requires that studies are adequately monitored. The sponsor should determine the appropriate extent and nature of monitoring. A study monitor, independent of Quotient Sciences, will be appointed to verify that the study is conducted in accordance with current GCP, regulatory requirements, the protocol, the Safety Management Plan and that the data are authentic, accurate and complete. A Monitoring Plan will be agreed and signed prior to start the screening activities.

The investigator agrees to receive visits from a study monitor and provide assistance to verify protocol implementation, source completion and transcription of data into the electronic case report form, document storage and AE reporting.

Quotient Sciences will extend the professional privilege of access to the subjects' clinical source documents to the study monitor, EC, regulatory bodies or other authorised personnel (eg auditor, sponsor representative) for the purposes of source data verification.

Following completion of the study both study related documents and subject data may be sent to the sponsor at a location outside of the UK where data protection laws differ. In the interests of confidentiality, subjects will not be identified on any such documents or data, and specific subject consent for such a disposition will be obtained.

20 Finance and Insurance

The sponsor (DNDi) has funded this study. A no-fault clinical trials insurance has been obtained by the sponsor. The sponsor insurance will compensate subjects in accordance with the Association of the British Pharmaceutical Industry Guidelines for Phase I Clinical Trials 2018 edition [10].

21 Publication

Please refer to the Clinical Trial Agreement information on publication.

Quotient Sciences shall have the right to publish the results of the research, subject to the sponsor's prior written consent, which shall not be unreasonably withheld or delayed. Following the receipt of such consent, Quotient Sciences shall submit a copy of the proposed publication to the sponsor who shall have 28 days in which to request amendments thereto which, to the extent that such proposed amendments are reasonable, Quotient shall be obliged to incorporate prior to such publication.

The sponsor undertakes that, prior to publication of any information, article, paper, report or other material concerning the research, it will submit a copy of such publication to Quotient Sciences who shall have 28 days in which to request amendments thereto which, to the extent that such proposed amendment are reasonable, the sponsor shall be obliged to incorporate prior to such publication. If requested to do so by DNDi, Quotient shall remove from the manuscript or presentation any Confidential Information prior to submitting the manuscript for publication or presentation. In addition, publication or presentation of the Results will be delayed for an additional ninety (90) days in the event that DNDi wishes to secure patent protection or to protect its proprietary rights and interests.

The Sponsor and Quotient agreed on following the rules described in the DNDi's Policy for Scientific and Clinical External Communications.

22 References

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[10] Guidelines for Phase I Clinical Trials. Association of the British Pharmaceutical Industry Guidelines. London, UK; 2018 Edition (publication date 29 May 2018).

[11] International Council for Harmonisation Good Clinical Practice (GCP) Guidelines approved by the Committee for Medicinal Products for Human Use (CHMP) on 17 Jul 1996, which came into force on 17 Jan 1997, updated Jul 2002, Integrated Addendum E6 (R2) dated 09 Nov 2016.

[12] The Medicines for Human Use (Clinical Trials) Regulations. Statutory Instruments 2004 No. 1031.

[13] The Medicines for Human Use (Clinical Trials) Amendment Regulations. Statutory Instruments 2006 No. 1928.

[14] The Medicines for Human Use (Clinical Trials) Amendment (No. 2) Regulations. Statutory Instruments 2006 No. 2984.

[15] The Medicines for Human Use (Clinical Trials) Amendment Regulations. Statutory Instruments 2008 No. 941.

[16] World Medical Association, Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects (and all subsequent amendments).

Appendix 1 Clinical Laboratory Parameters

Haematology	Clinical Chemistry	Virology	Urinalysis	Drugs of Abuse
Basophils Eosinophils Haematocrit (Packed Cell Volume-PCV) Haemoglobin Lymphocytes Mean Cell Haemoglobin (MCH) Mean Cell Haemoglobin Concentration (MCHC) Mean Cell Volume (MCV) Monocytes Neutrophils Platelet Count Red Blood Cell (RBC) Count White Blood Cell (WBC) Count **Coagulation Tests** Prothrombin Time Activated partial thromboplastin time (APTT) International Normalised Ratio (INR) Thrombin time	Alanine Aminotransferase (ALT) Albumin Alkaline Phosphatase Aspartate Aminotransferase (AST) Bicarbonate Bilirubin (Total) Bilirubin (Direct) (only if Total is elevated) Calcium Chloride Creatine Kinase (CK) Creatinine[a] Follicle Stimulating Hormone (FSH; post-menopausal female subjects only) Gamma Glutamyl Transferase (GGT) Glucose Glucose (Fasting) Potassium Phosphate (Inorganic) Protein (Total) Serum hCG (female subjects only) Sodium Urea	Hepatitis B Surface Antigen Hepatitis C Antibody HIV Antibody **Other safety parameters to be measured** Lactate dehydrogenase (LDH) Blood Urea nitrogen (BUN) Uric acid GLDH Cholesterol Troponin I (serum) Cystatin C (serum)	Bilirubin Blood Glucose hCG (female subjects only) Ketones Leukocytes Nitrites pH Protein Specific gravity Urobilinogen **At discretion of investigator based on urinalysis results** Microbiology Urine Microscopy	Amphetamines Barbiturates Benzodiazepines Cocaine Marijuana/Cannabis Methadone Methamphetamine/ Ecstasy Morphine/Opiates Phencyclidine Tricyclic Antidepressants

[a] Creatinine clearance will be estimated at screening for eligibility purposes and then at the time points indicated in Appendix 2, from serum creatinine using the Cockcroft-Gault equation.

Appendix 2 Study Flow Chart

Study Day	-28 to -2	Resident in Clinical Unit															
		-1	1											2	3	4	7 to 10 days post-dose
			Times After Dosing (h)														Follow-up visit
	Screening	Admission	Pre-dose	0	0.5	1	1.5	2	2.5	3	4	6	9	12	24	48	72[a]	
General Assessments																		
Informed Consent	X																	
Medical and Surgical History	X	X[b]																
Demographic Data	X																	
Hepatitis/HIV serology	X																	
Inclusion/Exclusion Criteria	X	X[b]	X[b]															
Weight and Height	X	X[c]															X[c]	
Vein Assessment	X																	
Carbon Monoxide Breath Test	X	X																
Drug Screen	X	X																
Alcohol Breath Test	X	X																
Serum Pregnancy Test[d]	X	X																
Urine Pregnancy Test[d]																		X
Randomisation[e]			X															
IMP Administration				X														
Safety Assessments																		
Physical Examination	X	X[f]													X[f]		X[f]	
Safety Labs[g]	X	X													X	X	X	X
Troponin I[h]		X									X		X		X	X		
FSH[i]	X																	
Urinalysis	X	X															X	X
Holter extracted ECGs[j]			←											→				
ECG[k]	X	X	X			X		X		X	X	X	X	X	X	X	X	X
Vital Signs[l]	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events	←																→	
Prior and Concomitant Medication	←																→	
Pharmacokinetic Assessments																		
Plasma Samples for DNDI-0690			X		X	X	X	X	X	X	X	X	X	X	X	X	X	
Urine Samples for DNDI-0690[m]			←											→				
Urine samples for exploratory markers[n]			X											X	X	X	X	

ECG: Electrocardiogram; FSH: follicle stimulating hormone; HIV: human immunodeficiency virus; IMP: Investigational Medicinal Product

[a] Discharge from clinical unit
[b] Update only
[c] Body weight only
[d] Female subjects only.
[e] Subjects will be randomised on the morning prior to dosing
[f] Targeted (symptom driven) physical examination
[g] Haematology, coagulation and clinical chemistry, LDH, BUN, Uric acid, GLDH, Cholesterol and Cystatin C at each time point. Virology at screening only. Creatinine clearance will be estimated at screening for eligibility purposes and at each time point, from serum creatinine using the Cockcroft-Gault equation.
[h] Time points may be altered based on emerging data from previous cohorts, see Section 8.2.1.
[i] Post-menopausal female subjects only
[j] Holter monitoring will begin at least 1 h prior to dosing and end 24 h post-dose after last PK time point. All ECG extractions will be taken in triplicate. Three sets of triplicate ECG extractions to be taken at pre-dose (for baseline). Holter extraction time points on Day 1 will be: pre-dose and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 9, 12 and 24 h post-dose.
[k] ECGs to be taken in triplicate at pre-dose (for baseline), all post-dose time points single ECG. An ECG can be repeated for any reason (technical in particular). The Principal Investigator or sub-investigator must double-check the results.
[l] Blood pressure and HR at every time point. Tympanic temperature at screening and pre-dose only.
[m] Urine will be collected for DNDI-0690 at the following intervals: Pre-dose, 0-12, 12-24, 24-48, 48–72 h post-dose. See Section 13.1.2 for more details.
[n] Urine samples for exploratory biomarkers for drug-related injury will be taken as an aliquot from PK urine collection at Pre-dose, 12, 24, 48 and 72 h post-dose. See Section 13.3.2 for details.