CLINICAL TRIAL PROTOCOL SYNOPSIS

A Phase 1, blinded, randomized, single centre, parallel-group, single-dose, dose-escalation, placebo-controlled study of the safety, tolerability, and pharmacokinetics of DNDI-6148 after oral dosing in healthy male subjects

Short title	DNDI-6148 Single Dose-Escalation in Healthy Male Subjects
Name of product	DNDI-6148
Drug Class	Benzoxaborole
Phase	Phase I, First in Human (FIM) study
Indication	Treatment of Leishmaniasis
Protocol Number	DNDi-6148-01
EudraCT	TBC
Sponsor	DND*i*, Chemin Louis Dunant, 15 1202 Geneva Switzerland Phone: +41 22 906 9230
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SAC approval	*TBD*
Clinical Trial Protocol Synopsis Version / Date	Version 1.0 / 12th June 2018

SYNOPSIS

Background Information and Trial Rationale	Leishmaniasis is generally seen as one of the most neglected tropical diseases and has strong links with poverty. It comprises a complex vector-borne disease, caused by more than 20 species of the protozoan genus *Leishmania* and ranging from localized skin ulcers to lethal systemic disease. Leishmaniasis is endemic in 101 countries/territories, with 350 million people at risk. Visceral Leishmaniasis (VL), also known as kala-azar, is caused by the protozoan parasites *Leishmania donovani* and *Leishmania infantum*, with a distribution in Asia, East Africa, Latin America and the Mediterranean region. It usually affects remote rural communities with difficult access to health care, and majority of cases are children. The parasites are transmitted through the bite of female phlebotomine sand flies and in the human host are obligate intracellular parasites of the reticuloendothelial system, surviving and multiplying in different macrophage populations. In patients who develop symptoms, presentation is insidious with development of splenomegaly, irregular fevers, anaemia or pancytopaenia, weight loss and weakness occurring progressively over a period of weeks or even months, and evolves toward death if untreated. Patients need life-saving treatment to be cured. There are important geographical features in VL: different parasites, response to drug treatment which varies between and within regions: higher dose regimens are required to achieve efficacy in the East Africa and Latin America as opposed to South Asia foci. Geographical variation has also been shown within East Africa. The differences in cure rate between Asia and Eastern Africa could be related to differences in parasite, host and/or drug exposure. The *L. donovani* population in Eastern Africa is genetically different from that in India, with high parasite population diversity observed in North region of East Africa. There is also evidence of different PK profiles of miltefosine in adult VL patients in Asia and Africa, with the former having higher drug exposure. Therefore, treatment regimens need to be adapted to the context of the disease in the regions, and clinical trial data cannot be extrapolated between regions. In the Indian sub-continent, a sharp decrease of number of VL cases occurred recently likely due to the conjunction of a successful elimination campaign, the natural fluctuating trend of incidence, vector control activities and improvement in the living conditions of the local population. It remains a major public health problem in East Africa (Ethiopia, Kenya, Somalia, Sudan, South Sudan and Uganda) and Latin America (mainly Brazil). **Existing treatment options, recent advances and unmet needs** Until recently, antimonial monotherapy for 20-30 days was the mainstay of treatment. However, in the last 15 years liposomal amphotericin B (AmBisome®), followed by paromomycin (PM) and miltefosine were developed or made available for use. In Asia, the 1st line treatment is a single dose of AmBisome®, whereas the combination paromomycin/miltefosine is 2nd line treatment, both with high efficacy and good safety profile. In Eastern Africa, Sodium Stibogluconate (SSG)&PM for 17 days has been recommended as a first line treatment by the WHO expert committee (2010). It is an improvement over the 30 days SSG monotherapy, but still requires double painful injections and carrying SSG toxicity. Patients with age above 45 years and with HIV co-infection are not suitable for this treatment, due to lower efficacy and higher mortality rates observed in these groups. Attempts to develop in Eastern Africa short course AmBisome® therapy and combination therapies with AmBisome® have failed due to poor and variable efficacy in the region. In Brazil, the guidelines are under review to replace the toxic meglumine antimoniate with AmBisome® as 1st line therapy. Despite the improvement and advances in VL therapy in the last decade, the current treatment options have limitations of being still toxic, not adapted to field conditions (most are parenteral drugs, with AmBisome® requiring cold chain), and some require long hospitalizations. In Africa regional meetings with VL experts, it has been defined as consensus that the

ideal treatment for East Africa should be a combination of oral drugs which is efficacious, safe and affordable.

DNDi priority is to eliminate the use of antimonials and develop a safe, effective, oral, short-course VL treatment that can be used at any health care level in all foci of the disease. If possible, DNDi will develop combination therapy based on newly developed NCEs. Innovative oral safe and highly efficacious combination treatments will have an impact in VL control, by delivering a therapy better adapted to the remote settings where VL transmission occurs.

Efficacy

DNDI-6148 is a 6-substituted benzoxaborole, which exhibits *in vitro* and *in vivo* activity against various strains of *Leishmania* parasites, including *L. donovani* and *L. infantum*, the causative agents of VL (see table below). DNDI-6148 by oral route is efficacious in mouse and hamster models of both acute and chronic VL infection.

No direct studies have been performed on the mode of action of DNDI-6148 at doses between 25 and 50 mg/kg/day.

Summary of *In vitro* Primary Pharmacodynamic activity of DNDI-6148*:

	Strain ID	Strain origin	DNDI-6148 Free Acid	DNDI-6148 Arginine monohydrate
			IC_{50} values (µM)	
L. donovani	MHOM/IN/80/DD8	India WHO reference strain	2.46	>10
	MHOM/ET/67/HU3	Ethiopia WHO reference strain	1.62 ± 1.07	Na
	MHOM/SD/62/1SCL2D	Sudan, WHO reference strain	0.15	Na
	GR265	Ethiopia, clinical isolate	na	8.36
L. infantum	MHOM/MA/67/ITMAP263	Morocco WHO reference strain	2.59 ± 1.66	2.35 ± 0.48
	MHOM/FR/09/LEM4038	France WHO reference strain	0.51 ± 0.11	na
	MCAN/DZ/2008/ENV48*	Algeria WHO reference strain	0.04 ± 0.03	na
	MHOM/FR/96/LEM3323 Cl4 PMM^	France WHO reference strain	0.69 ± 0.15	na

** methodology of experiment, more strains available in the IB*

ADME summary

DNDI-6148 is a low clearance compound, with moderate volume of distribution (1 to 2-fold total body water) and short to moderate elimination half-life in animals (in the range of 3-4 hours in rats and 4-7 hours in monkeys). All human metabolites detected *in vitro* are covered by toxicology species used as part of the regulatory preclinical package (rat and monkey).

The arginine monohydrate form of DNDI-6148 provided enhanced exposure (about 2-fold increase in all animal species tested except in hamster where exposure was similar) than the corresponding free acid form. DNDI-6148 arginine monohydrate was therefore selected for further development: all safety studies were performed with DNDI-6148 arginine monohydrate, and this is the form that is also intended to be administered orally to healthy volunteers.

Human PK parameters predictions based on animal data suggest that DNDI-6148 arginine monohydrate bioavailability should not be a limiting factor. The total efficacious daily human dose is estimated to be less than 500 mg (with reference to the active moiety DNDI-6148).

Safety pharmacology summary

There were no major safety concerns regarding the pharmacological effects of DNDI-6148 on respiratory and central nervous system functions assessed in rats up to 25 mg/kg.

The potential effects of DNDI-6148 on QT prolongation were assessed in *in vitro* hERG assays. DNDI-6148 did not significantly inhibit the amplitude of the current mediated by the hERG potassium channel *in vitro*, with IC_{50} value of >30.0 µM (17% inhibition at 30 µM) and up to the highest concentration tested, 45 µM (19% inhibition). As part of a four weeks GLP toxicity study in the conscious Cynomolgus, DNDI-6148 was assessed by telemetry at oral doses up to 15 mg/kg after 7 days. Results did not evidence any effects of DNDI-6148 on QTc nor any changes in the morphology of the ECG.

Regulatory toxicology studies

Toxicological safety assessment demonstrated that the toxicity profile of DNDI-6148 as determined in the repeat dose toxicity studies differed to some extent between the rodent and non-rodent species.

Doses of up to 25 mg/kg/day administered for four weeks did not induce any adverse effects in rats and 25 mg/kg/day was thus considered as the NOAEL in that species.

In the Cynomolgus monkey, the highest dose of 15 mg/kg/day resulted in a sacrifice for ethical reasons of one female before completion of the study, on Day 21. Blood samples were taken prior to necropsy and demonstrated parameters such as electrolytes, urea, creatinine and total protein being affected, however no macroscopic nor anatomical changes were observed. Histopathological evidences were reported in the adrenal glands and thymus and considered likely related to stress. Non-adverse stress related changes were also noted such as those observed in the large intestine (minimal acute inflammation in the colon and cecum), kidneys (slight bilateral cortical tubular dilatation) and sciatic nerve (minimal myelin vacuolation). At this dose level of 15 mg/kg, for all other male and female animals, there were no signs of overt toxicity in clinical observations or parameters monitored, including complete clinical pathology panel and histopathology. Systemic exposure (AUC0-24h) at NOAEL doses was 162 µg.h/mL in males (15mg/kg) and 64.8 µg.h/mL in females (5mg/kg) at steady state. Considering that a relationship between poor condition in one female that had to be sacrificed for ethical reasons and DNDI-6148 administration cannot be absolutely excluded, the NOAEL in Cynomolgus was established at 5 mg/kg/day for females and 15 mg/kg/day for males. The female NOAEL of 5 mg/kg/day was used as a basis of calculation of the starting dose in the phase I single ascending dose study.

Summary of Toxicokinetic and Pharmacokinetic parameters for DNDI-6148*

Species	Gender	Type of study	Dose PO (mg/kg/day)	AUC_{0-24} at steady state
Mice (acute model *L.infantum*)	**F**	Efficacy	25 (12.5 bid) /10 days 50 (25 bid) / 5 days i.e. minimal efficacy dose	
Mice	**F**	PK	50 (25 bid) /5 days	99.2 h*µg/mL
Hamster (chronic model *L.infantum*)	**F**	Efficacy	50 (25 bid) / 10 days i.e. minimal efficacy dose	
Hamster	**F**	PK	50 (25 bid) / 5 days	32.9 h*µg/mL
Rat	M	Toxicology PK	25 NOAEL 28 days	353 h*µg/mL
	F	Toxicology PK	25 NOAEL 28 days	347 h*µg/mL
Monkey	M	Toxicology PK	15 28 days (NOAEL for males only, not considered in dose calculations)	162 h*µg/mL
	F	Toxicology PK	5 NOAEL 28 days	64.8 h*µg/mL

** methodology of experiments, more doses and time-points available in the IB*

Genotoxicity

Standard *in vitro* and *in vitro* genotoxicity testing did not suggest any genotoxic/clastogenic potential on the nuclear genetic material for DNDI-6148. The drug is therefore not expected to pose a genotoxic risk for humans.

Starting dose and safety margin

Based on these results, DNDI-6148 is considered a promising candidate for clinical development as a new oral treatment for visceral leishmaniasis.

As mentioned above, the starting dose for this dose-escalation study has been selected based on the results of the 28-day toxicity studies in the rat and non-human primate, which indicated a NOAEL at 25 mg/kg/day in rats and 5 mg/kg/day in female non-human primates (most sensitive gender).

The Human Equivalent Dose (HED) is 4.03 mg/kg and 1.61 mg/kg respectively according to FDA *Guidance for Industry* (GUIDANC\5541fnlcln1) – July 2005, equivalent to 282 mg and 112 mg per 70 kg subject, respectively. 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 0.14 mg/kg to 0.16 mg/kg (10 mg per 70 kg subject) has been considered.

The proposed dose escalation is initially following a logarithmic scheme, then a progressively decreasing dose increment with dose level increase scheme.

The predicted human therapeutic dose is between 16 and 479 mg/subject for QD dosing,

	and 10 to 390 mg/subject for BID dosing (calculations based on allometric scaling, using Cynomolgus and rat data and combined with human *in vitro* hepatocytes data to calculate the theoretical clearance). The best and worst cases scenarios are based on the uncertainty of half-life in human, with a prediction ranging from 4 h to 11 h, in combination with an estimation of potentially efficacious dose, based either on C_{ave} *or* $C_{min.}$ The rationale for the highest scheduled dose of 500 mg in the Single Ascending Dose study is that it is just above the assumed higher human therapeutic dose level prediction derived from preclinical data. If required however, provided that safety stopping criteria are not met, a higher dose may be optionally considered and would be tested after substantial amendment of the protocol. Acute toxicity with single drug dosing is not anticipated to pose a risk of concerns to volunteers due to expected limited exposure. Once evaluated in male healthy volunteers, and provided no safety stopping rule has been met for a given dose, one additional cohort of healthy non-child-bearing potential women may be proposed for the same or a lower dose. All details would then be provided in a substantial amendment to the protocol. A compound from the same oxaborole family, SCYX-7158, developed for the treatment of human African trypanosomiasis, has been safely administered to 102 healthy subjects in France and is currently tested in a Phase II/III trial in Democratic Republic of Congo. **General objective** The present trial aims at evaluating the safety, tolerability and the PK parameters of the drug in humans after administration of a single dose by oral route. This will bring crucial information to evaluate if further development of this compound will be possible.
Trial Objectives	**Primary objective** To assess the safety and tolerability of DNDI-6148 after single oral doses administered as oral suspension of DNDI-6148 in healthy male subjects, compared to matching placebo. **Secondary objectives** - To determine $AUC_{0\text{-}24}$, AUC∞, AUC∞/D, Cmax, Cmin, Cave, Cmax/D for DNDI-6148 in plasma after single oral doses, administered as oral suspension in healthy male subjects. - To determine other PK parameters of DNDI-6148 after single oral doses administered as oral suspension of DNDI-6148 in healthy male subjects.

Trial Endpoints	

Efficacy: Not applicable.

Safety:

Safety assessments will include clinical laboratory assessments (including serum chemistry, hematology, and urinalysis – see page 14 for list of parameters), vital signs, body weight, ECGs (morphology, HR, PR, QRS, QT, QTcB, QTcF), both complete and symptom-based physical examinations, prior and concomitant medication reporting, and adverse event reporting.

ECGs will be conducted in singlicate for each timepoint, after a minimum of 10 minutes in resting position (in case of abnormality, a retest is allowed at a minimum of one minute interval between 2 subsequent ECG). Baseline assessment will be conducted in triplicate, at a minimum 1 minute interval between 2 subsequent ECG, and after a minimum of 10 minutes in resting position. The ECG recording will precede any vital signs collection and/or PK blood draw.

PK:

For each subject of each dose level, blood will be collected at the following time points: pre-dose, and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 9.0, 12.0, 24.0, 48.0, 72.0 h post-dose.

Urine will be collected during the following intervals: pre-dose, then]0-12],]12-24],]24-48],]48-72] h post-dose.

Plasma:

Based on the plasma concentration time data (collected after administration of DNDI-6148 suspension), the following PK parameters will be calculated:

- Main DNDI-6148 PK parameters: AUC∞, Cmax
- Exploratory DNDI-6148 PK parameters: $AUC_{0\text{-}24}$, AUC∞/D, Cmax/D, Cmax,norm, Cmin, Cave, Tmax, t½, MRT, CL/F, AUC∞,norm, AUC t, AUC t,norm, Vz/F
- Other DNDI-6148 parameters: λz, AUC t-∞, points terminal
- Exploratory identification of DNDI-6148 metabolites at the highest dose level reached

Urine:

The amount and concentration of DNDI-6148 will be measured. The appropriate specific PK parameters to be calculated will be decided according to the concentration.

The following parameters will be calculated from urine data for DNDI-6148:

- **$Ae_{(0\text{-}t)}$** Total amount excreted over 24 h and 72 h (*i.e.* t = 24 or 72).
- **fe** The fraction of the dose excreted in urine over 24 h and 72 h.
- **CL_r** The renal clearance of DNDI-6148

Pharmacodynamic:

QT evaluation will be based on 24h 12-lead Holter recording on Day 0, and ECGs. Holter recordings will be archived. Analyses will be conducted at the end of the trial if decision to progress to Multiple Ascending Dose (MAD) study is taken.
The choice of timing for extraction of records from the Holter will be done after PK analysis. At each blood sampling time-point, the Holter-extracted triplicate parameters (RR, PR, QRS and QT) will be averaged to obtain on single value per time point. On day 0, three pre-dose triplicates will be extracted and averaged to establish the baseline value.

Changes from baseline with the recorded Holter (Day 0) will be calculated for each parameter:

- Categorical analysis for QT, QTcI, QTcP, QTcF, QTcB, ΔQTcI, ΔQTcP, ΔQTcF and ΔQTcB according to pre-defined thresholds ECG,

	• ECG morphological analysis. The pre-defined thresholds for the categorical analysis will be as detailed below. – For maximum actual values: • QTc interval > 450 ms • QTc interval > 480 ms • QT/QTc interval > 500 ms – For maximum time-matched changes from baseline: • ΔQTc interval > 30 ms • ΔQTc interval > 60 ms All parameters and their changes from baseline will be summarized by dose regimen and time point. Placebo group extracts will be used for the double-delta analyses of QT.
Trial Design	Randomized, blinded, placebo-controlled, single centre, single ascending dose study with DNDI-6148 administered as an oral suspension. Bioanalysis (in plasma and urine) will be performed in open conditions. Decision on dose escalation will be taken in blinded conditions, based on safety interim report and DNDI-6148 plasma exposure. The study is proposed in healthy, male Caucasian subjects. Currently there are no data to suggest that DNDI-6148 is sensitive to ethnic factors, according to criteria in ICH Guideline E5 '*Ethnic Factors in the Acceptability of Foreign Clinical Data*'. As per the ICH E5 guidelines, knowledge of the pharmacokinetic and pharmacodynamic properties and the translation of these to clinical efficacy and safety are required to assess if DNDI-6148 will be sensitive to ethnic factors. A cohort of healthy female subjects of non-child bearing potential may be included at a later stage in the study (by substantial amendment). Same for specific ethnic origin cohort.

Main Entry Criteria Inclusion Exclusion	**Inclusion Criteria** Subjects will be required to satisfy the following criteria: 1. Male healthy Caucasian subjects 18 to 50 years of age. Optionally, after further evaluation during the study, at the sponsor's discretion other ethnic groups may be recruited, 2. Male subjects with a body mass index (BMI) calculated as weight in kg/ (height in m^2) from 18 to 30.1 kg/m^2 at screening, 3. Able to communicate well with the Investigator and research staff and to comply with the requirements of the entire study, 4. Provision of written informed consent to participate as shown by a signature on the volunteer consent form, after reading the information and consent form, and after having the opportunity to discuss the trial with the investigator or his delegate, 5. Light smokers (less than 5 cigarettes per day) or subjects who are non-smokers. No smoking (or use of smoking substitute *e.g.* nicotine patch) is permitted from screening throughout the study, 6. Normal arterial blood pressure (BP) and pulse rate or, if abnormal, considered not clinically significant by the Principal Investigator. These will be measured after resting for 10 min. 7. Registered in agreement with the applicable law on biomedical experimentation. **Exclusion Criteria** All the subjects included in the study must not meet any of the following exclusion criteria. Excluded will be subjects: 1. Who on direct questioning and physical examination have evidence of any clinically significant acute or chronic disease, including known or suspected HIV, HBV or HCV infection, 2. Who previously received DNDI-6148, or who participated in another clinical trial within 3 months prior and during the study, or 5-times the half-life of the drug tested in the previous clinical trial, whichever is longer (time calculated relative to the last dose in the previous clinical trial), 3. With any clinically significant abnormality following review of pre-study laboratory tests (i.e. ASAT, ALAT, ALP, Creatinine and Urea must be within normal ranges), vital signs, full physical examination and ECG, 4. Who are within the exclusion period defined in a National Register for Healthy Volunteers of the Ministry of Health, 5. Who forfeit their freedom by administrative or legal award or who were under guardianship, 6. Who are unwilling to give their informed consent, 7. Who have a positive laboratory test for hepatitis B surface antigen (HbsAg), or anti-HIV 1/2 or anti- HCV antibodies, 8. Who have a history of allergy, intolerance or photosensitivity to any drug, 9. Who have a history of serious allergy, asthma, allergic skin rash or sensitivity to any drug, 10. Who have a history of additional risk factors for "Torsades de Pointe" (e.g., heart failure, hypokalemia, family history of Long QT Syndrome). 11. Patients with rare hereditary problems of fructose intolerance, glucose-galactose malabsorption or sucrase-isomaltase insufficiency, 12. Who are known or suspected alcohol or drug abusers (more than 14 units of alcohol per week, one unit = 8 g or about 10 mL of pure alcohol), 13. Who drink more than 8 cups daily of beverage containing caffeine,

	14. Who have a positive laboratory test for urine drug screening (opiates, cocaine, amphetamine, cannabis, benzodiazepines), 15. Who have undergone surgery or have donated blood within 12 weeks prior to the start of the study, 16. Who have any clinical condition or prior therapy which, in the opinion of the Investigator, made the subject unsuitable for the study, 17. Who had surgery (e.g. stomach bypass) or medical condition that might affect absorption of study drug taken orally, 18. Who had febrile illness within 1 week before the start of the study, 19. Who has regular daily consumption of more than one liter of xanthine-containing beverages, 20. Who has regular daily consumption of more than 5 cigarettes daily, or use more than 3 grams (1/8 ounce) of tobacco, 21. Who used a prescription medicine during the 28 days before the first dose of trial medication or use of an over-the-counter medicine (including antacid drug, with the exception of acetaminophen (paracetamol)), during the 7 days before the first dose of trial medication, 22. Who use dietary supplements or herbal remedies (such as St John's Wort) known to interfere with the CYP3A4 and/or P-gp metabolic pathways during the 28 days before the first dose of trial medication. 23. Grapefruit should also be avoided during the 7 days before the first dose of trial medication. The inclusions criteria will be amended prior to running cohort 9.
Study Duration	Screening will occur within 28 days before admission to the research unit. One single oral administration per subject Duration of clinical phase by subject (apart from the screening period): 4 days (from Day -1 evening to Day 3 morning) 9 cohorts of 8 subjects are planned with dose escalation every 1.5 to 2 weeks, for a total of 5 months of recruitment. For sentinel dosing, see below.

Study treatments

Oral suspension of DNDI-6148 has been chosen as formulation for the SAD study. DNDI-6148 will be pre-weighted in closed bottles and a suspension will be prepared extemporaneously by the pharmacist prior to administration to volunteers. The powder will be suspended in ORA-Sweet® vehicle, a maximum of 24 hours prior to dosing. Volume to be administered will vary from 4 to 25 ml per subject.

Table 1. Description of the 9 cohorts

Cohort	Dose		Increase from the previous dose
	mg	mg/kg*	
1	10	0.14	
2	20	0.29	+ 100%
3	40	0.57	+ 100%
4	80	1.14	+ 100%
5	160	2.29	+ 100%
6	260	3.71	+ 63%
7	380	5.46	+ 46%
8	500	7.14	+ 32%
9	Maximum of 500	Maximum of 7.14	Optional

*** assuming a 70-kg person**

Table 2.

Cohort	Treatment and dose (randomization)
1 (8 subjects)	Single oral administration of DNDI-6148, 10 mg on Day 1 or placebo (6/2)
2 (8 subjects)	Single oral administration of DNDI-6148, 20 mg on Day 1 or placebo (6/2)
3 (8 subjects)	Single oral administration of DNDI-6148, 40 mg on Day 1 or placebo (6/2)
4 (8 subjects)	Single oral administration of DNDI-6148, 80 mg on Day 1 or placebo (6/2)
5 (8 subjects)	Single oral administration of DNDI-6148, 160 mg on Day 1 or placebo (6/2)
6 (8 subjects)	Single oral administration of DNDI-6148, 260 mg on Day 1 or placebo (6/2)
7 (8 subjects)	Single oral administration of DNDI-6148, 380 mg on Day 1 or placebo (6/2)
8 (8 subjects)	Single oral administration of DNDI-6148, 500 mg on Day 1 or placebo (6/2)
9 (8 subjects)	Duplication of one cohort as required (maximum of 500 mg on Day 1 or placebo (6/2) or cohort conducted in female subjects or in African-origin subjects or Indian-origin subjects

At each dose level, subjects will be split at least in two sub-cohorts: first sub-cohort will be one subject under active, one subject on placebo. Second sub-cohort will be composed of 5 subjects under active, one subject under placebo. The 2nd sub-cohort will be dosed after a minimal 24 hours of safety surveillance will be available from the first 2 subjects. Decision of dosing of the 2nd sub-cohort will be under the responsibility of the investigator. Any concern should be shared with the Sponsor prior to next dosing.

Starting with the lowest dose, each of the subsequent escalating doses will be administered only if the preceding dose was safe and well tolerated. Decision to escalate the dose will be taken during the meeting of the Safety Review Committee described below after reviewing complete blinded PK data and safety data collected over the 3 days following study drug administration:

- Adverse events (AEs),
- Safety ECG,
- Vital signs,
- Laboratory tests.

The 3-day surveillance period has been defined using the predicted half-life of the drug (i.e. sufficient time to determine the terminal half-life and maintain the clinical and biological surveillance up to final drug elimination). Predicted half-life of DNDI-6148 calculated by several methods lies between 7 to 11 hours, five half-lives correspond to 35 hours to 55 hours, while the volunteers will remain under surveillance for 72 hours. In the worst-case scenario of a half-life of 12h, five-half-lives i.e. 60 hours would also be covered by those 3-day surveillance.

The study will proceed to the next dose level only if the safety results are acceptable, as agreed by the Safety Review Committee. A minimum of 6 subjects per cohort with complete safety data available will be required to take a decision to proceed to the next dose level (i.e. minimum of 4 subjects under active).

Preliminary plasma PK analysis up to the H72 timepoint will be necessary to escalate the dose from 1st to 2nd cohort for the 6 subjects under active, then preliminary PK analysis up to H48 for a minimum of 4 subjects under active for the subsequent cohorts.

One cohort can be duplicated to enlarge the number of subjects in one sub-group in case of need.

Recruitment has been limited to Caucasian population to insure homogeneity of assessments within a cohort. A specific cohort including other ethnic groups (African's origin and/or Indian's origin) may be considered at the Sponsor's discretion to check the safety and PK in this specific population. This would correspond to the duplication of a dose-level well tolerated in the study. This will either be presented through an amendment or an adaptive design option. Similarly a cohort of healthy female subjects of non-child bearing potential may be included at a later stage in the study (by substantial amendment).

Trial stopping criteria

The trial will be stopped if either of the following occurs:

• a 'serious' adverse reaction (SAR) (i.e. a serious adverse event (SAE) considered at least possibly related to DNDI-6148) in one subject; or

• 'severe' non-serious adverse reactions (AR) (i.e. severe non-serious adverse events considered as, at least, possibly related to DNDI-6148) in two subjects in the same cohort, independent of within or not within the same system-organ-class.

If, after an internal safety review, it is appropriate to restart the trial, a substantial amendment will be submitted to the Health Authorities and Ethics Committee. The trial will not restart until the amendment has been approved by the Health Authorities and Ethics Committee.

Dose escalation stopping criteria and within a cohort

A dose level will not be repeated, or exceeded, if the results of safety tests give the Sponsor or Investigator cause for concern, or if one of the following dose escalation stopping criteria is met:

- any of the trial stopping criteria (see above) are met.
- the Investigator considers the dose level to be not well tolerated.
- predicted mean plasma concentrations in the subjects at the next scheduled dose level exceed or equal: Cmax: 7.3 µg/mL, AUC(0-24): 65 µg·h/mL (based on the NOAEL level in Cynomolgus toxicology studies).

The scheduled dose of DNDI-6148 may be reduced if, for example, the results of safety tests give any cause for concern, or tolerability is poor. If AEs occur that cause mild or moderate discomfort but do not in any way threaten the health of the subject, that dose level may be repeated, with the aim of further exploring the relationship between dose/exposure and AE. If, in the judgement of the Safety Review Group, 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 adverse events (AEs) and dose (or plasma concentration) of drug.
Statistics Sample size Randomisation Summary of analysis	**Number of Subjects Planned: 72** Subjects will be randomized 6 active/2placebo in 2 sub-cohorts (1 active/1 placebo, then 5 active/1 placebo) DNDi, investigators and subjects will be blinded to treatment allocation. Site pharmacist will be open to treatment allocation.

Table 2- Schedule of events

	SCREEN[1]	Cohort 1 to 8 (or 9)				
		Days				
		-1	0	1	2	3
Hospitalization[2]		←	—	—	—	→
Inclusion/exclusion criteria	X	X[3]				
Demographic data	X					
Medical history / previous medication	X	X[3]				
Urinary drug screen	X	X				
Alcohol breath test	X	X				
Serology	X					
Informed consent	X					
Study drug administration			X			
Clinical examination	X	X		X[6]		X
Vital signs[4]	X	X	X[5]	X[6]		X
12-leads ECG[14]	X	X[13]	X[5]	X[6]		X
Holter 24-hours recording[15]			X			
Clinical chemistry & hematology	X	X	X[7]	X[6]		X
Urinalysis	X		X[7]	X[6]		X
Body weight	X					X
PK blood sampling			X[8]	X[6]	X[9]	X[10]
PK urine sampling			X[11]	X	X	
AEs & concomitant medication recording	←	—	—	—	—	→

Study plan: details of Day 1 up to 24 h post-dose

	Cohorts 1 to 8 (or 9)												
			Post-dose										
	Pre-dose	T0	30 min	1 h	1h30	2 h	2h30	3 h	4 h	6 h	9 h	12 h	24 h
Study drug administration:		X											
Vital signs	X			X	X	X	X	X	X	X		X	X
12-leads ECG[13]	X	X		X		X		X	X	X	X	X	X
PK blood sampling	X		X	X	X	X	X	X	X	X	X	X	X
Blood sampling for metabolites identification				X[12]									

1 Within 28 days before admission to the clinical unit
2 Evening (about 6.00 *p.m.*) on Day -1, discharge on Day 3 morning after completion of the EOS assessments
3 Recheck only
4 Blood pressure (supine and standing) and oral temperature
5 Pre-dose, 1, 2, 3, 4, 6, 9 and 12 h post-dose
6 24 h post-dose
7 Pre-dose
8 Pre-dose, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 9 and 12 h post-dose
9 48 h post-dose
10 72 h post-dose
11 Pre-dose and]0-12];]12-24];]24-48];]48-72] post-dose
12 Blood sampling from cohort 2 and subsequent cohorts at predicted Tmax timepoint (one timepoint between 1 to 6 hours post dose, synchronised with regular PK blood sampling time)
13 Triplicate ECG at minimum 1 min interval between 2 subsequent ECG
14 ECG recording will precede any vital signs collection and/or PK blood draw. Retest allowed for all singlicates at minimum 1 min interval between 2 subsequent ECG.
15 Holter recording should start minimum 1 hour prior to dosing

Laboratory tests

The following clinical laboratory tests will be performed at the pre-study screening and at the follow-up examination except serology and urine drug screening.

Haematology and Coagulation Parameters:

- Hemoglobin,
- Red blood cell (RBC),
- Haematocrit,
- Mean corpuscular volume (MCV),
- Mean corpuscular hemoglobin (MCH),
- Mean cell hemoglobin concentration (MCHC),
- White blood cell (WBC) including differential,
- Platelet counts,
- Reticulocytes count,
- Haptoglobin,
- Prothrombin time (PT), activated partial thromboplastin time (aPTT) and International normalized ratio (INR).

Clinical Chemistry (Serum/Plasma):

- Alkaline phosphatase (ALP),
- ALAT,
- ASAT,
- Gamma–glutamyltranspeptidase (GGT),
- Creatine phosphokinase (CPK),
- Total bilirubin,
- Total protein,
- Creatinine,
- Fasting glucose,
- Urea,
- Electrolytes (Na+, K+, Cl-)

Urine will be examined by a standard stick test for:

- Glucose,
- Ketones,
- Density,
- Occult blood,
- pH,
- Proteins,
- Leukocytes.

Planning Information

Study Timelines

Final protocol available	*August 2018*
Study treatment supply available	*July 2018*
FSFV	*October 2018*
Duration of recruitment period	*5 months*
Duration of follow-up period (if applicable)	*3 days per subject*
LSLV	*Q1 2019*
Interim analysis	*Not planned*
Final study report	*Q2 2019*

STUDY SCOPE

Target countries	*France*
Enrolment target	*72 subjects*
Number of sites	*One single center*
Number of subjects per site	*72 subjects*
DSMB involvement	*No DSMB planned, dose escalation meetings after each cohort gathering DNDi team members, investigators and external consultants*
Partners involvement	*CRO for bioanalytics (in urine and plasma): SGS Wavre* *CRO for central ECG reading* *CRO for EU QP release: Pharmaceuticals solutions Ltd, UK.* *CRO for CRF, Data Management, Statistics and Clinical Study Report*
Other study special needs	ORA-Sweet® to be sourced by the Phase I CRO

Study Treatments Supply

<table>
<tr><td>Study treatments</td><td>
Bottles of pre-weighted powder of DNDI-6148 arginine monohydrate (corresponding to 60 and 600 mg free acid equivalent) and corresponding placebo.

To be used as a suspension: to be resuspended extemporaneously with ORA-Sweet® (30 mL per bottle), then volume corresponding to the dose to be administered to be measured by the pharmacist at the CRO centre using appropriate precision syringes (volume from 4 to 25 ml/subject), if possible opaque to minimize risk of unblinding (DNDI-6148 is not sensitive to light).

<table>
<tr><td>Strengths (mg) of DNDI-6148 in bottle</td><td>60</td><td>600</td></tr>
<tr><td>Concentration (mg/ml)*</td><td>2</td><td>20</td></tr>
<tr><td></td><td></td><td></td></tr>
<tr><td>Dose escalation (mg)</td><td colspan="2">Volume to administer (ml)</td></tr>
<tr><td>10</td><td>5</td><td></td></tr>
<tr><td>20</td><td>10</td><td></td></tr>
<tr><td>40</td><td>20</td><td></td></tr>
<tr><td>80</td><td></td><td>4</td></tr>
<tr><td>160</td><td></td><td>8</td></tr>
<tr><td>260</td><td></td><td>13</td></tr>
<tr><td>380</td><td></td><td>19</td></tr>
<tr><td>500</td><td></td><td>25</td></tr>
</table>
*(30 mL working volume bottles)

250 ml of mineral still water to be administered after dosing.

Manufactured in India by Syngene and QP released by a UK-based CRO.
</td></tr>
<tr><td>Labelling instructions</td><td>Follow Annex 13 European GMP</td></tr>
<tr><td>Other information</td><td>Not applicable</td></tr>
</table>