DNDi
Drugs for Neglected Diseases *initiative*

CLINICAL TRIAL SYNOPSIS

A phase II, multicentre, randomized, two-arm blinded study to assess the efficacy and safety of two LXE408 regimens for treatment of patients with primary visceral leishmaniasis

Short title	LXE408 Phase 2
Name of product(s)	LXE408 and Liposomal amphotericin B (AmBisome®)
Drug Class	Proteasome inhibitor – LXE408 Macrocyclic, polyene antifungal antibiotic – AmBisome®
Phase	Phase II
Indication	Primary visceral leishmaniasis
Clinical Trial Protocol Number	DNDi-LXE408-01-VL/ CLXE408A12201R
Sponsor	DND*i*, Chemin Camille-Vidart, 15, 1202 Geneva, Switzerland Phone: +41 22 906 9230
Coordinating Investigator	[redacted]
Principal Investigators	[redacted]

Background Information and Trial Rationale	LXE408 is a first-in-class parasite-selective inhibitor of the kinetoplastid proteasome with potent and uniform anti-parasitic activity against all kinetoplastid parasites, including Leishmania species causing visceral leishmaniasis (L. donovani and L. infantum), as well as parasites causing Chagas disease (T. cruzi) and human African trypanosomiasis (T. brucei). Safety data in healthy volunteers suggest that LXE408 is safe and tolerated at all doses tested (up to 600 mg single dose and 600 mg multiple dose). **Purpose of the Phase 2 clinical trial:** This study aims to assess the efficacy, safety and PK profile of LXE408 in primary VL patients in India. A SDA arm will be included as a calibrator arm, to have efficacy and safety data in similar patient population and timeframe of study conduct. If LXE408 is proven to be efficacious with a favorable safety profile in future confirmatory studies, this therapy may be an alternative therapy to the use of SDA. It would be an attractive short-course oral option that can be used at any health care level (including primary health care setting closer to the communities affected by the disease) in all foci of the disease. This will improve and simplify current case management and aims to reduce time between onset of symptoms and access to treatment, therefore reducing morbidity and mortality for the patient, and also reducing transmission and contributing to disease control and elimination. In addition, exploratory biomarkers will also be assessed in this phase 2 study, aiming to identify potential markers of cure/failure at the end of treatment, and prognostic markers of relapse.

Trial Objectives	**Primary objective** • To assess the efficacy of a 7-day and a 14-day treatment course of LXE408 in adult patients with primary VL at Day 28 **Secondary objectives** The following secondary objectives will be assessed in adult VL patients: • To assess the safety and tolerability of LXE408 • To assess the PK of LXE408 • To assess the efficacy of LXE408 at Day 180 • To assess the effect of LXE408 on blood and tissue parasite clearance • To assess PK and PD relationships • To describe the safety, efficacy, PK and PD for AmBisome® **Exploratory objectives** • To explore efficacy, safety, and PK of LXE408 in adolescent patients • To explore the role of the blood transcriptome as a predictive biomarker for efficacy • To assess the impact of LXE408 treatment on patient symptoms and health related quality of life (QoL)
Trial Endpoints	**Primary endpoint** The primary efficacy endpoint is the proportion of patients with initial cure at study Day 28. Initial cure: cure at Day 28, defined as clinical improvement of VL, absence of parasites in the spleen or bone marrow (microscopy), and no rescue therapy on or before Day 28. Definition for clinical improvement of VL at Day 28 includes all of the following parameters: - absence of fever attributed to VL - spleen size reduction of any magnitude in relation to baseline - any increase (if abnormal at baseline) or no worsening* (if normal at baseline) in haemoglobin, WBC, and platelet values in relation to baseline *in the absence of any other intercurrent illness which, in the opinion of the investigator, is the cause of the worsening. **Secondary endpoint(s)** • Safety and tolerability: - Frequency and severity of treatment-emergent adverse events (TEAEs), SAEs, and AEs requiring treatment discontinuation. - Descriptive statistics and frequency of physical exam

	abnormalities, vital signs, ECG abnormalities, safety laboratory assessments including clinical chemistry, hematology and urinalysis results up to and including end-of-study (EOS) visit. - All-cause mortality at days 28 and 180 and mortality not associated with VL at days 28 and 180. • Pharmacokinetics: - The following PK parameters will be determined on D1 and D7 for LXE408: Cmax, Tmax, AUCtau, CLss/F - The following PK parameters will be determined on D1 for AmBisome®: Cmax, AUC0-24h, and AUC0-infinity • Efficacy: - At Day 28 for Ambisome®: proportion of patients with initial cure at Day 28 (as described above) - At Day 180 for LXE408 and Ambisome®: proportion of patients with definitive cure at Day 180 Definitive cure described as initial cure at Day 28, no requirement for rescue treatment throughout the study, no death associated to VL and absence of any clinical parameters of VL at D180. • Additional parasitological endpoints: - Blood parasite clearance over time, as measured by quantitative polymerase chain reaction (qPCR) from blood samples, at screening, D1, D3, D5, D7, D10, D14, D28, D56, and at any suspicion of relapse during the trial - Proportion of patients with a positive loop-mediated isothermal amplification (LAMP) from blood samples, at baseline, D28, D56, and at any suspicion of relapse during the trial - Tissue parasite loads, as measured by qPCR from tissue samples (spleen or bone marrow) collected at baseline, Day 28, and at any suspicion of relapse during the trial - Proportion of patients with a positive LAMP from tissue samples at baseline, D28, and at any suspicion of relapse **Exploratory endpoint(s)** • Descriptive statistics of efficacy, safety, and PK parameters for adolescent patients included in LXE408 arms • Host biomarkers for patients included in LXE408 arms only: differentially expressed genes and pathways in peripheral blood at D3, D7, D14, D28, D56, and at relapse, compared to baseline (D1),

	by study drug, treatment outcomes, and drug related (S)AEs. • Health-related QoL endpoints for patients: - Change in WHOQoL-BREF scores from baseline to D14, D28, and D180/EOS. - Change in EQ-5D-5L scores from baseline to D14, D28, and D180/EOS. - PGIC scale at D28 and D180/EOS
Trial Design	This is a phase II, multicentre, randomized, two-arm blinded study with an open label calibrator arm in adults and adolescents (≥12 years) with confirmed primary VL. The study will enroll and randomize approximately 95 adults in a 2:2:1 ratio to one of three treatment arms: - LXE408 300 mg PO once daily for seven days (followed by 7 days of placebo) (38 patients), - LXE408 300 mg PO once daily for 14 days (38 patients) - Standard of care (AmBisome® 10 mg/kg IV single dose (SDA), 19 patients) Both investigator and patients will be blinded for the LXE408 investigational arms, which will reduce bias in the clinical assessment of safety and efficacy outcomes. However, the SDA arm will remain unblinded, given the significant differences between the investigational regimen (oral daily administration) vs SDA, which is a one-time injectable regimen (2h IV infusion). Each arm will consist of a screening period of up to 7 days, a 28-day treatment period (which encompasses the different treatment durations and the test of cure visit at Day 28), and a follow-up period from Day 29 to Day 180. All patients will be hospitalized for approximately 21 days, from the first day of screening period to the Day 14 visit, when they are expected to be discharged. They will return to the study sites at the scheduled Day 28 visit (±1 day), and during the follow-up period they will return for the Day 56 (± 7 days) and EOS visit at Day 180 (± 14 days). In addition, during follow-up between Day 56 and Day 180, the study team will contact the study patients by phone on a monthly basis to check on their well-being and any reappearance of VL symptoms. A check on the PK parameters will be performed once 10 patients complete the treatment, and subsequently on PK and qPCR parameters once 20 patients included in the intensive LXE408 sampling have completed the treatment. Trends in safety and clinical parameters will also be monitored regularly. One interim analysis (IA) will be performed when approximately 40% of the total number of planned patients have either completed the Day 28 assessment or discontinued the study due to failure before D28.

	After this IA, recruitment in LXE408 arm may be expanded to a sub-group of approximately 10 adolescent VL patients, upon recommendation from an independent Data Monitoring Committee (IDMC). In addition, the inclusion of a new arm with adjusted daily dose/regimen may also be considered. **Assessments:** Efficacy assessments will include clinical parameters (fever clearance, spleen and liver sizes, hematological parameters), and parasitological evaluation at baseline, at Day 28 (test of cure) and at any suspicion of relapse during the trial. Parasitological assessment will be done through spleen or bone marrow aspirate and microscopic examination. Spleen aspirate is preferable, due to its higher sensitivity. Safety assessments will include physical examinations, vital signs, clinical laboratory evaluations (hematology, clinical chemistry, urinalysis), ECG, adverse event (AE) and serious adverse event (SAE) monitoring. Pharmacokinetics Pharmacokinetics of LXE408: Intensive PK will be performed for the first 20 patients included in LXE408 arms. The sampling time will be as follows: • D1 and D7 at: 0, 1, 2, 3, 4, 8, hours. • Troughs (pre-dose samples) would be on days 2, 3, 4, 5, 8, 10 and day 14. Once the first 10 patients, and subsequently 20 patients, included in LXE408 intensive PK sampling complete the treatment (Day 14), PK samples will be analyzed to assess if the observed exposure is within the predicted efficacious range, as per PK model; and to confirm the PK sampling schedule proposed for the remaining patients in the trial. For all remaining adult patients, sparse sampling will be performed. The drug concentration measurements will be performed on D3, D5, D7, D10 and D14 (pre-dose samples). In addition, a blood sample for drug levels will be collected for any SAE and AEs that lead to treatment discontinuation. The extra blood sample will be collected as soon as possible, if no other scheduled PK sample is planned. A PK sample will also be collected if treatment is discontinued due to an initial failure (i.e., during the 14 days of treatment, prior to initiating rescue therapy). Furthermore, upon recommendation from the IDMC, intensive PK (as described above) will be performed for the 10 adolescents recruited in the LXE408 arms. The adolescent group is an additional subgroup to the study.

	Pharmacokinetics of AmBisome: PK of amphotericin B in VL patients treated with SDA is also included in the present study, as this data is not available in the literature. PK/PD relationship will also be explored for SDA arm. Total amphotericin B concentration will be measured in the first 10 VL patients treated with SDA in the following time-points: D1: 0, at the end of infusion and at 0.5, 1, 2, 4, 8, 12 and 22 hrs after the end of infusion. The remaining 9 patients will have PK samples collected at time-points 0 and at the end of AmBisome infusion. <u>Parasite dynamics and biomakers</u> Furthermore, qPCR and LAMP in blood will be performed to assess parasite clearance over time, before, during and after treatment The first 20 patients with LXE408 intensive PK sampling will have matching daily PD samples to be collected for qPCR, *i.e.,* at screening, D1, D2, D3, D4, D5, D7, D8, D10 and D14. Samples will be analysed in batches. Once the first 10 and subsequently 20 patients allocated to LXE408 intensive sampling complete treatment, qPCR will be analysed to monitor trends in parasite clearance, and to confirm the schedule of qPCR sampling for the trial. For the remaining patients: - qPCR will be performed at screening, D1, D3, D5, D7, D10, D14, D28, D56 and at any suspicion of relapse during the trial. - LAMP will be performed at baseline, D28, D56 and at any suspicion of relapse during the trial. qPCR and LAMP will also be performed in tissue at screening, D28 and at any suspicion of relapse. Extra blood samples will be collected at baseline (D1), D3, D7, D14, D28, D56 and at relapse to assess host biomarkers for patients included in LXE408 arms only. <u>Patient Reported Outcomes</u> Patients reported outcomes using WHO-QoL-BREF (among adult patients) and EQ-5D-5L questionnaires) will be collected at baseline (Day 1), D14, D28 and D180. Patient Global Impression of Change (PGI-C) will be completed at D28 and D180. <u>Rescue therapy:</u> Rescue treatment will be indicated for patients who do not respond to the study treatment: cases of initial failure (during treatment up to Day 28 test of cure) and cases of relapse during follow-up. All cases of treatment failure must be documented with a positive parasitological diagnosis.

Entry Criteria Inclusion Exclusion	Patients who fulfill all inclusion criteria and do not present any of the exclusion criteria are eligible for enrollment in this study. **Inclusion criteria**: • Male and female patients ≥ 18 years (at the time of the screening visit) who are able to comply with the study protocol. Following a favourable interim analysis result, patients ≥12 <18 years will also be enrolled in the trial • Patients for whom written informed consent has been obtained (if aged 18 years and over) or signed by parent(s) or legal guardian for patients under 18 years of age. In the case of minors, assent from the child also needs to be obtained • Primary symptomatic VL (defined as typical parameters including, but not limited to, fever for > 2 weeks, weight loss, and splenomegaly) • Visualization of *Leishmania* amastigotes by microscopy in tissue samples (spleen or bone marrow) **Exclusion criteria:** • Clinical signs of severe VL (jaundice, spontaneous bleeding, edema, ascites, coma, organ failure) • Laboratory abnormalities including ALT/SGPT > 3 times ULN, total bilirubin > 1.5 times ULN, creatinine >1.5 times ULN, amylase or lipase > 1.5 times ULN, haemoglobin < 6 g/dL or other clinically significant abnormal laboratory parameters which, in the opinion of the investigator, may indicate severe VL • Patients with history of previous leishmaniasis and confirmed relapse • Patients with para-kala-azar dermal leishmaniasis • Patients with severe malnutrition (for children ≥12-<18 years: BMI-for-age WHO reference curves by sex, z score < -3; for adults ≥18 years: BMI < 16) • History of congenital or acquired immunodeficiency, including positive HIV (test at screening) • Known hypersensitivity to amphotericin B deoxycholate or any other constituents of AmBisome® • Concomitant infections such as tuberculosis, severe malaria, or any other serious underlying disease that may interfere with the disease

	assessment (e.g., cardiac, renal, hepatic, haematologic, and pancreatic) • Infection with hepatitis B (HBV) or hepatitis C virus (HCV). A positive HBV surface antigen (HBsAg) test, or if standard local practice, a positive HBV core antigen test, excludes a subject. Patients with a positive HCV antibody test should have HCV RNA levels measured. Patients with positive (detectable) HCV RNA should be excluded. • Pregnant or nursing (lactating) women • Women of childbearing potential who do not accept to have a pregnancy test done at screening and/or who do not agree to use highly effective contraception while taking the investigational drug and for 5 half-lives or 5 days, whichever is longer, after stopping the investigational drug. • Sexually active males unwilling to use a condom during intercourse while taking the investigational drug and for 5 half-lives or 5 days, whichever is longer, after stopping the investigational drug.
Study Duration	Subject participation in the trial will consist of a 7 day screening period, 28 days treatment period (treatment durations of 1 day for SDA, 7 or 14 days for LXE408, with a test of cure at Day 28) and follow-up period until Day 180, therefore a total of approximately 6 months duration. Recruitment for the entire trial is expected to take 12 months assuming that 20% of all VL patients will meet the eligibility criteria. Therefore, study duration from first patient first visit (FPFV) to last patient last visit (LPLV) is expected to take approximately 18 months. Total study duration from FPFV to key results shall last approximately 24 months.
Study treatments	Patients will be assigned to one of the treatment arms in a ratio of 2:2:1 : • Arm 1 (up to 38 patients): oral LXE408 300 mg q24h for 7 days followed by 7 days of placebo q24h • Arm 2 (up to 38 patients): oral LXE408 300 mg q24hr for 14 days • Arm 3 (19 patients): Standard of Care AmBisome 10 mg/kg i.v. infusion q24hr, one single dose (SDA) LXE408 should be administered in the morning and preferably before the first meal. An additional sub-group of at least 10 adolescents will be included if favorable safety and no futility is confirmed by the IA, in order to obtain efficacy, safety and PK data in this population: • Arm 1 (at least 5 adolescents): oral LXE408 300 mg q24h for 7 days followed by 7 days of placebo q24h • Arm 2 (at least 5 adolescents): oral LXE408 300 mg q24hr for 14 days Note: the dose for adolescents may be adjusted based on PK data from adults in the IA.

Statistics	Based on the early stage of development, the hypothesis in terms of efficacy will therefore be less stringent than that defined for the overall program (and future confirmatory studies): for this study, we will implement a futility threshold of 60% with a desirable cure rate of at least 80%. SDA arm is included as a calibrator arm, to have efficacy data in similar patient population and timeframe of study conduct, and to minimize confounders in drug related AEs due to underlying disease. Hence descriptive statistics on efficacy and safety will be summarized for SDA arm, but no statistical comparisons will be performed between LXE408 arms and SoC arm. Up to 95 adults (85 evaluable patients) will be randomised to one of three treatment arms in a ratio of 2:2:1 (38 LXE408 arm 1: 38 LXE408 arm 2: 19 SDA) to provide ≥80% power, using a one-sided type I error ≤0.05 per comparison to detect an increase in cure rate (CR) from a minimal or "undesirable" rate of 0.6 to a desirable one of 0.8. A drop-out rate of 10% is assumed for this sample size. An initial check on the PK and PD parameters will be performed once 10 patients included in the intensive LXE408 PK sampling complete treatment. Trends in safety and clinical parameters will also be monitored regularly. A planned interim analysis will be conducted after approximately 16 subjects per LXE408 arm and 8 subjects in the SDA arm have either completed the D28 assessment or discontinued the study due to treatment failure before D28 (approximately 40% of the planned total number of patients having evaluable data at D28). The unblinded safety, efficacy and PK interim data will be reviewed by an independent data monitoring committee (DMC). Based upon safety, efficacy, and PK data at IA, the inclusion of a new arm with an adjusted daily dose and/or regimen may be considered. The sample size in this new arm will be adjusted if any of the assumptions change. All patients having evaluable data at the time of the IA will be included. Patients who are defined as treatment failures, study-drug related deaths, or deaths associated to VL before Day 28 will be considered as non-responders. If the decision at the time of the IA is to continue the study (favorable safety and efficacy), the recruitment will be expanded to a sub-group of at least 10 adolescents (≥12 years) to obtain preliminary efficacy, safety, and PK data in this population (randomize 5 adolescents/ LXE408 arm or 10 adolescents in 1 arm if the other arm is stopped after IA).