DNDi
Drugs for Neglected Diseases *initiative*

CLINICAL TRIAL PROTOCOL SYNOPSIS

An Open Label, Phase III, Randomized Controlled, Multicentre Non-Inferiority Trial to Compare Efficacy and Safety of Paromomycin and Miltefosine Allometric Regimens with Sodium Stibogluconate and Paromomycin Combination for Treatment of Primary Visceral Leishmaniasis (VL) Patients in Eastern Africa

Name of product(s)	Miltefosine (Impavido) (MF) Paromomycin (PM) Sodium Stibogluconate (SSG)
Drug Class	Alkylphosphocholine for MF Aminoglycoside for PM Pentavalent antimonial for SSG
Phase	Phase III
Indication	Primary Visceral Leishmaniasis
Protocol Number	DNDi-MILT/PM-01-VL
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Clinical Trial Protocol Synopsis Version / Date	Final 1.0, 3 Feb 2017

SYNOPSIS

Background Information and Trial Rationale	Visceral leishmaniasis (VL) is a parasitic disease caused by *Leishmania.* It is fatal if not treated. The disease is characterized by fever, weight loss, hepatosplenomegaly, lymphadenopathy, anaemia, leucopenia and thrombocytopenia. Globally there are 200-400,000 estimated new cases of VL occurring annually and 90% of these occur in six countries: Bangladesh, Brazil, Ethiopia, India, Sudan and South Sudan. In Eastern Africa, VL is caused by *Leishmania donovani,* and it affects mainly children. Eastern Africa is expected to become the VL focus region for global VL control, following significant VL elimination efforts in Southeast Asia. WHO revised the recommended VL treatment for Eastern Africa in 2010 from sodium stibogluconate (SSG) monotherapy (30 days treatment) to a combination of SSG and PM administered for 17 days, following a phase III trial conducted in the region. The efficacy of SSG-PM combination was 91% in the intention to treat population using a complete case analysis, which was non-inferior to a 30-day SSG monotherapy. This new treatment is an improvement over the 30-day SSG monotherapy, but a few important drawbacks/limitations still preclude its general use. Although the treatment regimen is shorter, it still requires 17 days of two separate painful injections, necessitating patients to be hospitalized during the whole treatment period. In addition, there are life-threatening toxicities associated with the use of antimony-based treatments such as SSG. These include cardiotoxicity, hepatotoxicity and pancreatitis. AmBisome® (liposomal amphotericin B) is used as 2nd line drug for rescue treatment and for specific target populations such as pregnant women, severe disease or HIV co-infection. The need for cold chain, high cost and the administration by well trained personnel limit the widespread use of AmBisome®. Therefore, there is a need to explore alternatives that are efficacious, safe, ideally of short duration, affordable and suitable to be used in remote areas where VL occurs. In this context, an oral efficacious and safe treatment would be more adapted to field conditions and would allow shorter hospitalization time. In order to respond to this need, upstream pre-clinical research at DNDi has been focused on identification of orally available new chemical entities (NCEs) that meet these requirements. Promising NCEs are expected to transition from pre-clinical to clinical phase in 2017-2018, bringing new opportunities for innovation in VL therapy. However, the full development of a new oral treatment is not expected before 2022-23. As we wait for the NCEs, new opportunities with currently available compounds should be assessed to improve on current treatment options, with the main aim to replace the toxic and patient-unfriendly SSG treatment-component. Currently, miltefosine (MF) is still the only oral drug available for VL

	treatment. As a phosphorylcholine ester of hexadecanol, it is a membrane-active alkylphospholipid, which interferes with the membrane lipid metabolism (*e.g*. de novo synthesis of phosphatidylcholine). It acts through numerous interactions with cell membrane components and cell signaling pathways. It also induces apoptosis through the PI3K-Akt pathway and possibly through mitochondrial dysfunction. Numerous immune-modulatory actions of MF have been described, potentially contributing indirectly to its mechanism of action *in vivo*. MF has been extensively used in Asia for VL treatment as monotherapy for 28 days. Treatment of up to 12 weeks is recommended for PKDL in Asia. It is well tolerated, the main side effects are vomiting and nausea, and transient increases in liver enzymes and creatinine. Majority of the events are mild and do not require treatment discontinuation. The main limitation of MF is its teratogenicity, which requires contraception in female patients of childbearing age during treatment and after treatment for at least 5 months. Furthermore, in the US Product Information there is a warning on potential male fertility toxicity, which has been observed in rats. The rationale for this warning was based on findings observed at the Human Equivalent Dose (HED) calculations. However, when alternative analysis on risk of fertility impairment was done based on exposure data (PK) in animals rather than HED, a better safety margin could be derived of 2-3 fold in male rats and at least 3.5 fold in female rats, taking into consideration the lowest dose where reversible findings were observed in animals. This is in line with retrospective data from VL patients treated with MF in India showing 69% of proven fertility, vs 52% in the Amphotericin B control arm (assessments were done between 11 and 57 months after start of MF treatment). In another study in Columbia, sperm tests were performed in CL patients, resulting in no clinically relevant effect on sperm viability or spermatogenesis. However, these studies had methodological issues and were not considered appropriate to rule out the risk on male fertility. MF was evaluated as monotherapy and in combination with Ambisome in a phase II clinical trial in Kenya and Sudan (LEAP0208). None of the treatment regimens reached the predefined acceptable satisfactory efficacy level of $\geq$ 90% (point estimate) to be taken forward in a Phase III trial to compare with SSG+PM: Ambisome + MF (10 days regimen) showed a cure rate of 77% (95% CI: 64-90%), and MF as monotherapy (28 days regimen) had 72% (95% CI: 60-85%) efficacy at 6 months follow up. Although not statistically significant (study was not powered for this comparison), there was a clear trend of a poorer efficacy in children (7-12y) as compared to adults (13-60y) in the trial, especially in the MF monotherapy arm (59.1% vs 86.2%, *p= 0.061*). The conventional MF dose linearly based on weight (mg/kg), calculated

	as 2.5 mg/kg/day, did not provide similar drug exposure in children as compared to adults. This is in line with previous published MF PK data from India and Nepal (Dorlo JID 2014 and Dorlo AAC 2012). MF has a long half-life (7 days), and keeps accumulating over the treatment duration to eventually approach steady-state at the 4th week of treatment. In the LEAP0208 study, for 28 days treatment, the mean end-of-treatment MF concentration was 22.1 µg/mL (IQR 16.8-29.1 µg/mL) vs 30.2 µg/mL (IQR 24.7-36.3 µg/mL) (difference 37%, p<0.001), respectively for patients weighing <30 kg vs patients ≥30 kg. To overcome this under-dosage of children, an allometric dosage for paediatric VL has been assessed in the LEAP 0714 trial in Kenya and Uganda. This MF dosing is based on an allometric algorithm by fat free mass. In practice, individual weight, height and sex are used to determine the dose, which entails administering a relatively higher mg/kg/day dose in patients with lower body weight compared to patients with a higher body weight. The 28 days regimen of MF allometric dose showed a cure rate of 96.7% (95% CI: 82.8-99.9%) at Day 28 and a cure rate of 90.0% (95% CI: 73.5-97.9%) at 6 months follow up in a population of 30 patients aged 4 to 12 years. These results showed that efficacy level was increased in children treated with the allometric dose as compared to the conventional dose, reaching similar efficacy observed in adults in the LEAP0208 trial (*i.e.* 86.2%). In general, MF was well tolerated and compliance in the hospital setting was 100%. There was no treatment discontinuation due to drug related adverse events. Two SAEs were reported (anaemia and transfusion reaction), none of them related to MF, which is consistent with the good safety profile of this drug. PK analysis was performed to further characterize the drug exposure and PK properties of MF comparing allometric versus historic data on the conventional regimen. The overall MF exposure (area under the concentration-time curve from zero to infinity) was 12% higher compared to conventional dosing in the same pediatric age group. MF seemed to accumulate faster in LEAP0714 with the allometric dosing in the first week of treatment, resulting in a higher drug exposure during the first half of the treatment: e.g. day 7 MF concentrations were generally much higher for LEAP0714 (median 5,880 ng/mL) than for LEAP0208 (median 2,670 ng/mL), although this difference was not significant due to high variability. While total miltefosine exposure was thus increased, children treated with MF allometric dosing did not achieve the expected exposure i.e. similar to adults treated with conventional therapy of 2.5 mg/kg/d for 28 days. A potential reason for this was an apparent stagnation of the MF accumulation between day 14 and day 21 for 40% of the LEAP0714 patients, after which MF concentrations rose again between day 21 and day 28. Nevertheless, the variability in MF concentrations and overall exposure was higher for the linear dosing regimen than for the allometric regimen. This had an effect on the proportion of patients reaching a PK target threshold, with

	less patients being low exposed. It was previously shown that the duration someone was above 10x EC50 (17.9 µg/mL) as measure of MF PK was associated with a lower probability of disease relapse and subsequent rescue treatment. 15% of paediatric patients enrolled in LEAP0714 did not reach the 17.9 µg/mL threshold (10xEC50), compared to 29% for LEAP0208 in the paediatric subset. This in combination with the 12% overall higher total exposure to miltefosine could possibly explain the improved clinical efficacy observed in children treated with the allometric regimen. Based on the results of this trial, MF is an oral drug that remains an attractive option for combination with other drugs, and for children (< 30Kg), the allometric dosing is a better regimen, both in terms of drug exposure and therapeutic outcome, while maintaining a good safety profile. Paromomycin (PM) is a broad spectrum aminoglycoside antibiotic effective against a wide range of bacteria and protozoa. It has been extensively studied in Asia, where the 10-day combination of PM with MF is currently one of the treatment options used for VL, with a very safe profile and high efficacy of 98% in the phase III trial and also in the effectiveness study in field conditions. The most frequently reported AEs related to PM are injection site pain, transient mild or moderate increases in alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, creatinine and bilirubin enzymes, and reversible ototoxicity. PM has been well studied in Eastern Africa during the development of the combination SSG-PM. The initial PM monotherapy arm was 15mg/kg/day IM for 21 days, based on satisfactory efficacy data from Asia. The overall efficacy at 6 months was 63.8% (81/127 cured) with high variability across treatment sites (14.3 to 96.6%). The study arm was stopped and a dose-finding study was implemented in Sudan to assess the efficacy of PM monotherapy given as 15mg/Kg/day for 28 days (extended duration) or 20mg/Kg/d for 21 days (increased daily dose), with an efficacy at the 6-months follow-up of 81% (17/21, 95%CI 58-95%) and 80% (16/20, 95%CI 56-94%) respectively. The gain in efficacy was similar either by extending duration or increasing PM daily dose, suggesting that it is driven by the overall drug exposure. The protocol was amended, to replace the PM monotherapy arm with the regimen of 20mg/kg/d IM for 21 days. The overall efficacy of the 20mg/kg/d regimen at 6 months was 84.3% (167/198 cured), with more homogeneous profile across sites (80% to 93.3%). Doses of 15mg/kg/d and 20mg/kg/d presented very similar safety profiles. Increasing the PM dose to 20mg/kg/d did not increase the incidence of liver or kidney toxicity, ototoxicity or serious adverse events. Therefore, both regimens could be considered for combination with MF. Considering that the PM efficacy is driven by overall exposure, and both regimens are safe, the 20 mg/kg/d regimen is a better option as it allows for shorter treatment duration. Intramuscular PM

	can be administered at primary health care level, requires minimal training of health personnel and the drug can be stored at room temperature. Moreover, it is the cheapest of currently available anti-leishmanial drugs. The current study aims to determine if combined PM and MF treatment regimens are non-inferior to the SSG-PM combination currently used as first line treatment in Eastern African VL patients. If proven safe and efficacious (non-inferior), this therapy can be an alternative over the use of SSG as a component of the current treatment. It would minimize the number of injections, remove the direct antimonial toxicity, could be an attractive option for children that represents a high proportion of the population at risk and the elderly who are most at risk of SSG-induced toxicity, therefore potentially improving the overall benefit/risk profile of VL treatment. Another important advantage of this combination is the reduced cost and increased suitability to be used in remote areas. The strategy to combine one parenteral drug for a short period (PM 20 mg/kg/d for 14 days) with an oral drug for the same or longer duration (MF allometric dosing for 14 or 28 days) is to reduce as much as possible the hospitalization period, while extending the treatment to achieve appropriate exposure and satisfactory efficacy levels. The 14 days therapy is more attractive, as it ensures compliance to both drugs at hospital setting, and it will be cheaper; whereas the 28 days therapy has a higher chance to achieve satisfactory efficacy not inferior to SSG-PM. Testing both regimens in parallel will allow better quantification of the pharmacokinetic-pharmacodynamic relationship of the regimen, given the expected wide range of total MF exposure. In addition, the 28 day MF treatment arm allows for assessment of compliance to treatment in a non-hospital setting. Ultimately, if both arms of this trial demonstrate non-inferiority to SSG-PM, the 14 day regimen will be preferable to be taken forward for future standard of care or alternative treatment to SSG-PM. Data will be presented to MoH and regulatory authorities in the region as evidence to support policy change for the national control programs.
Trial Objectives	<u>Primary objective</u>: To compare the efficacy of two combination regimens of PM (14 days) and MF (14 or 28 days) with the standard 17-day course of SSG-PM for the treatment of primary VL patients in Eastern Africa <u>Secondary objective:</u> • To compare the safety of two combination regimens of PM (14 days) and MF (14 or 28 days) with the standard 17-day course of SSG-PM

	• To describe the pharmacokinetic (PK) profiles of PM and MF (14 days and 28 days regimen) in primary VL patients • To evaluate parasite clearance in each arm as indicated by direct microscopy and quantitative polymerase chain reaction (qPCR) • To assess the relationship between PK and PD measurements (parasitological and clinical outcome) • To assess compliance to MF treatment in an outpatient setting
Trial Endpoints	Primary endpoint: *Definitive cure* - cure at 6 months follow up defined as absence of clinical signs and symptoms of VL at D210 and no requirement for rescue treatment during the trial (e.g. no initial treatment failure or no relapse). Secondary endpoint: • **Safety:** Frequency of SAEs and AEs requiring treatment discontinuation. Frequency and severity of adverse events from the start of treatment through the last visit, at D210. • **Efficacy**: *Initial cure* - cure at the end of treatment (Day 28), defined as recovery of clinical signs and symptoms; absence of parasites (microscopy) and no rescue treatment administered up to and including Day 28. *Probable cure* - absence of clinical signs and symptoms of VL at D56 and no prior requirement for rescue medication. • **Pharmacokinetics**: Total and partial blood plasma exposure to PM and MF (bioanalysis performed by a validated LC-MS/MS assay), defined as the area under the concentration-time curve. For MF, AUC will be measured during treatment and until the last point of follow-up. For PM it will be based on full curves both on the first day of treatment (day 1) and the last day of treatment (day 14). • **Pharmacodynamics:** Blood parasite clearance over time (qualitative and quantitative), as measured by qPCR from blood samples, from baseline until day 210, and at any suspicion of relapse during the trial. Tissue parasite loads, as semi-quantified by microscopy and qPCR from tissue samples collected at baseline, at the end of treatment (D28) and at any suspicion of relapse during the trial.

	• Compliance to MF treatment in an outpatient setting will be assessed through patients' hospital records history, drug accountability and PK measurements.
Trial Design	This is an open label, Phase III, randomized, controlled, parallel arm multicentre non-inferiority clinical trial to compare the efficacy and safety of two combination regimens of PM and MF with SSG-PM for the treatment of primary adult and children VL patients in Eastern Africa. The study will be conducted at 6 LEAP sites: Kimalel and Kacheliba in Kenya, Amudat in Uganda, Doka and Um El Kher in Sudan and Gondar in Ethiopia. Assessment of recruitment strategy in Baringo county where Kimalel is located is ongoing, including involvement of Chemolingot as a possible satellite site or a clinical trial site. The 2 treatment regimens to be tested are: • **Arm 1**: Paromomycin 20 mg/kg/d IM for 14 days combined with oral miltefosine allometric dosing for 14 days • **Arm 2**: Paromomycin 20 mg/kg/d IM for 14 days combined with oral miltefosine allometric dosing for 28 days The reference arm is the current standard treatment for VL: • **Arm 3**: Sodium Stibogluconate 20 mg/kg/day IM combined with Paromomycin 15 mg/kg/day IM for 17 days For patients weighing < 30 kg, an easy-to-use table with allometric dosing scheme by weight, height and sex will be provided to the investigators to define the exact daily dose to be administered. For patients weighing ≥ 30 kg, the allometric dose will correspond to the conventional dose in mg/kg. Therefore, in order to simplify the dose calculation, patients weighing ≥ 30 to 44 kg will receive 100 mg/day and patients ≥ 45 kg will receive 150 mg/day. The target population will be VL patients from 4 to 50 years old in order to cover both paediatric and adult population. The limit of 50 years for inclusion is due to higher mortality rate and lower efficacy observed in patients > 50y when treated with SSG-PM in a recent pharmacovigilance program. PM and MF will be administered together, both starting at D1. PM dosage will be 20 mg/kg/d for 14 days administered once-a-day intramuscularly, whereas MF allometric dose will be administered orally b.i.d. for 14 days in arm 1 and for 28 days in arm 2. Patients will be hospitalized for 14 days of PM and MF treatment for both arm 1 and arm 2. MF treatment will start at the same time as PM treatment and for arm 2 it will continue on an out-patient basis until completion of the 28 days treatment. During hospitalization, compliance to treatment is assured. During this period, patients or parent/guardian will be instructed on MF

	treatment, the daily dose (morning and afternoon), and the administration with food to avoid or minimize any vomiting. Patients in arm 2 will be discharged after 14 days in hospital with clear instructions on how to continue MF treatment, and return for Day 28 visit. Compliance during the 14 days treatment outside the hospital will be assessed by collecting the empty blisters pack at the day 28 visit and checking for any unused drug. A daily diary will be provided to these patients in order to guide them in their treatment schedule at home. Any episode of vomiting reported by the patient will be captured by the clinician at day 28 visit. In case of multiple episodes of vomiting or any other adverse events, patients will be instructed to come to the hospital for an assessment and management in an unscheduled visit. Compliance will be further assessed through cross-check with PK data. SSG-PM combination therapy will be administered for 17 days according to routine VL treatment guidelines. Patients will remain hospitalized for the entire duration of SSG-PM treatment. Please see the complete list of assessments to be performed in Table 1 – Schedule of Events. All patients will have pharmacokinetics measurement of MF at D28 and D56 in order to measure end of treatment MF concentration and verify adherence to the treatment. In addition, a subset of patients in Kenya and Sudan will be asked to participate to intensive sampling for both PM and MF PK, defined as the intensive cohort: 40 in each MF/PM combination arms, with selection of 20 pediatric (≥7 to ≤12 yrs) patients and 20 adults. Patients who agree to be part of the intensive cohort will be asked to provide a separate written informed consent. The intensive cohort will also have additional qPCR blood samples collected (see Table 2 - Schedule of PK/PD events – Intensive cohort). **Safety** The adverse events reporting period will begin upon subject enrolment in the trial (after signature of informed consent) and will end at the end of the subject participation in the trial (D210 follow-up visit). According to safety profile of the drugs, safety assessments will include patient history, physical exam, laboratory tests (complete blood count (CBC), ALT, AST, bilirubin, creatinine, and albumin), audiometry test and ECGs. Adverse Events will be assessed by temporality, causality, severity, relationship to study treatment, seriousness and outcome. Severity of AEs will be graded using the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE version 4.0). The AE data will be coded using MedDRA dictionary.

Main 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:** • Patients with clinical signs and symptoms of VL and confirmatory parasitological microscopic diagnosis • Patients aged 4 to ≤ 50 years who are able to comply with the study protocol • Patients for whom written informed consent has been obtained (if aged 18 years and over) or signed by parents(s) or legal guardian for patients under 18 years of age. In the case of minors, assent from the children also needs to be obtained as per each country regulatory requirements **Exclusion criteria:** • Patients who are relapse cases • Patients who have received any anti-leishmanial drugs in the last 6 months • Patients with severe malnutrition (for children aged <5 years: weight-for-height WHO reference curves by sex, z score <-3; for children 5-18 years: BMI-for-age WHO reference curves by sex, z score < -3; for adults ≥19 years: BMI < 16) • Patients with positive HIV diagnosis • Patients with previous history of hypersensitivity reaction to any of the study treatments • Patients with previous history of cardiac arrhythmia or with a clinically significant abnormal ECG • Patients suffering from a concomitant severe infection such as TB or any other serious underlying disease (cardiac, renal, hepatic) which would preclude evaluation of the patient's response to study medication • Pregnant or lactating women • Female patients of child bearing age who do not accept to have a pregnancy test done at screening and/or who do not agree to use contraception from treatment period until 5 months after the end of treatment • Patients with haemoglobin < 5g/dl • Patients with WBC < 1 x 10^3/mm^3 • Patients with platelets < 40,000/mm^3 • Patients with abnormal liver function (ALT and AST) tests of more than three times the normal range. • Patients with bilirubin more than 1.5 times the upper normal range • Patients with serum creatinine above the ULN for age and sex.

	• Patients with clinical signs of severe VL disease such as jaundice and bleeding • Patients with pre-existing clinical hearing loss based on audiometry at baseline • Patients who cannot comply with the planned scheduled visits and procedures of the study protocol
Study Duration	Each patient's participation in the study will be for approximately 7 months. This will consist of baseline assessments, treatment period (14, 17 or 28 days) and 6 months follow-up. Recruitment for the entire trial is expected to take 14 to 17 months assuming that 30 to 35% of all VL patients will meet the eligibility criteria. Therefore, study duration (first patient in to last patient, last visit) is expected to take approximately 24 months. Taking into account the analysis and reporting period, the study shall last at most 31 months. In order to meet the recruitment target, all LEAP sites will be prepared to initiate the trial. Once EC/regulatory approvals are obtained in the country, recruitment shall start, and other sites to be added *ad hoc* as approvals are obtained.
Study treatments	1. Paromomycin 20 mg/kg/d IM q.d. for 14 days combined with oral miltefosine dosing b.i.d. for 14 days 2. Paromomycin 20 mg/kg/d IM q.d. for 14 days combined with oral miltefosine dosing b.i.d. for 28 days 3. Sodium Stibogluconate 20 mg/kg/day Intravenous / Intramuscular (IV/IM) q.d. and Paromomycin 15 mg/kg/day IM q.d. for 17 days MF dosing will be defined as follows: - Patients <30 kg: the allometric MF daily dose will be calculated according to subject's weight, height and sex - Patients ≥ 30 to 44 kg: 100 mg/day - Patients ≥ 45 kg: 150 mg/day

Pharmacokinetics	The pharmacokinetics of both PM and MF will be determined using validated LC-MS/MS bioanalytical assays (PM to be validated). The pharmacokinetics of both drugs will be determined in plasma, unless a dried-blood-spot method is developed for PM prior to initiation of this trial. All patients will have pharmacokinetics measurement of MF at D28 and D56 in order to verify adherence to the treatment. In addition, intensive sampling for both PM and MF PK will be performed in a subset of patients defined as the intensive cohort: 40 in each MF/PM combination arms, with selection of 20 pediatric (≤12 yrs) patients and 20 adults. Enrollment in this intensive cohort will be limited to specific sites located in Sudan and Kenya. These sites will be selected based on their experience, equipment and resources available. Since previous trials (LEAP0714 and LEAP0208) have raised questions on both the (reduced) absorption rate, but also extent of absorption (bioavailability), of MF in East African VL patients, denser sampling is required in this trial than previously performed for MF pharmacokinetics. Provided the PK nonlinearities observed in pediatric patients receiving the allometric MF dosing (LEAP0714), the inclusion of children in this PK substudy is important and therefore the aim is to have equal numbers of children and adults included in the PK substudy, to allow for comparison. In order to avoid multiple blood sampling in young children, only children from ≥7 years of age and weighing more than 20 kg will be included in the PK sampling schedule. To reduce the number of total samples/blood volume per patient, a mixed sampling approach will be performed. Patients will be randomly allocated to sparse sampling schemes to allow for a pooled PK analysis. PM PK assessments will be performed only in the intensive cohort (subset of 40 patients in each MF/PM combination arms) at Screening, D1 and D14. Each patient will be allocated to one of the following sparse PK sampling schedules: - D1: either 1, 2, 4, 24h or 1, 2, 8, 24h - D14 (arm 1 and 2): either 0, 1, 2, 4, 24h or 0, 1, 2, 8, 24h MF PK assessments will be performed in all subjects at D28 and D56 to confirm adherence to treatment. Subjects who consent to participate to the intensive cohort will have MF PK assessments performed at Screening, D1, D7, D14, D28 and D56 in both arms and an additional assessment will be done at D21 in arm 2 only. In the intensive cohort, MF PK sample at D1 and D14 will be collected at the same time points as PM PK at 0, 1, 2, 4, 8 and 24h (according to the sparse sampling scheme the patient is allocated to).

Statistics	**Sample size calculation**
Sample size	Samples size calculation is based on the efficacy (definitive cure) at 6 months (D210) which is the primary endpoint and main parameter of interest. Assuming an expected efficacy at the end of 6 months follow-up of 93% for the tested combination and 91% for the reference arm SSG-PM, a non-inferiority margin of 7%, and 1.25% alpha (one-sided adjusted for multiplicity), the sample size required to reach a power of at least 80% per comparison of interest (each investigational arm vs SSG-PM) is 173 patients per arm rounded to 175. In order to adjust for potential lost to follow-up (LTFU), the sample size will be 192 patients per group, a total of 576 patients in the trial. Sample size calculation is based on a single look on un-blinded data. This means that no interim analysis (IA) will be performed. An IA could have been considered in order to select the arm with satisfactory efficacy. However, based on the expected enrollment rate, by the time the number of patients required for the IA will complete the 6 months follow-up (primary efficacy analysis), most of the patients will have been already enrolled in the trial. Considering the time needed for interim report writing and alpha adjustment needed for the IA, it is not considered worthwhile to perform an IA.
Randomization	**Randomization** Randomization will be centralized. Subjects will be randomized using block randomization. Blocks of different sizes (6, 9 or 12 subjects) will be used in random order, with an allocation ratio of 1:1:1. DNDi Data Center in Nairobi, Kenya will conduct data management, analysis and reporting of the trial.
Summary of analysis	**Summary of analysis** The following patient populations will be defined for analysis: • Modified Intention To Treat (mITT) or Full Analysis Set (FAS): All randomized patients receiving at least one dose of treatment. In case of error of treatment allocation, the actual treatment received will be used in the analysis. Modified ITT set of patients will be used in the primary efficacy analysis and in safety analysis. • Set of completers: subset of patients belonging to the FAS, who attend the 6-months follow-up visit. This set will be

used in a sensitivity analysis of the primary efficacy analysis. This analysis will be based on the definitive cures.

- Per protocol set of patients: subset of patients belonging to the FAS and who are free from major protocol violations. Major protocol violations are any violation which might bias the result of the non-inferiority test. Major violations will be defined a priori in the statistical analysis plan. They will be identified and documented during the blind review of data. This set of patients will be used in the primary sensitivity analysis.

Primary endpoint: Based on a ternary outcome

- Definitive cure or treatment success:

- absence of signs and symptoms of VL at D210,
- no rescue medication at any time up to D210 (included).

- *Treatment failure*:

Patient who requires rescue treatment due to any of the following:

- Initial failure;
- occurrence of adverse events that leads to treatment discontinuation
- relapse at any time;

or

- death during treatment period or death during follow-up period that is related to VL
- Lost to follow-up at D56 or before.

- *Unconfirmed status at D210*: Patient who

- did not fail at day 28 (initial cure) and at day 56 (probable cure) and
  did not relapse before D210 (no rescue medication, no signs and symptoms of VL before day 210) and
  did not attend day 210 visit

Definitions:

Initial failure: patient who did not respond to the treatment and required rescue therapy within the 28 days treatment period; or patient who had a positive parasitology (by microscopy) at the D28 assessment.

Relapse: patient who had responded to treatment and had a negative parasitology at the D28 assessment, but who presents signs and/or symptoms of VL during follow-up, with VL confirmed by presence of parasites in a parasitological investigation.

Secondary efficacy endpoints:

Initial cure - cure at the end of treatment (Day 28), defined as recovery of clinical signs and symptoms; absence of parasites (microscopy) and no rescue treatment administered at Day 28 or before.

Probable cure - absence of clinical signs and symptoms of VL at D56 and no prior requirement for rescue medication.

Primary Efficacy Analysis

The primary efficacy analysis will be based on

- the modified intention to treat population (mITT or FAS);
- the primary endpoint (cure at D210);
- the primary imputation approach (see below) and the primary test of non-inferiority, which is the Blackwelder test.
- primary comparisons: PM + MF 14 days vs SSG-PM and PM + MF 28 days vs SSG-PM.

The overall significance limit will be 0.025 (one-sided). The Holm procedure will be used to adjust alpha for multiplicity of testing.

If the non-inferiority test is significant the test of superiority will be performed.

Handling of unconfirmed status

The handling of unconfirmed status can be based on imputing a failure to all unconfirmed status and in that case the success rate is probably underestimated (ITT worst-case scenario). Another approach is to consider all cases of probable cure at D56 as definitive cure at D210. This is equivalent to the use of last observation carried forward approach or even considering an absence of proven failure as a success. This approach is probably overestimating the success rate. A third intermediary approach consists in imputing a probability of success which is neither a failure (Imputed probability of success = $IP_S = 0$) nor a success ($IP_S = 1$) but an imputed probability of success ($0 < IP_S < 1$). This is the basic idea leading to the primary imputation method.

The estimated probability of success (P_S) is equal to 1 minus the probability of relapse (P_R) between day 56 and day 210. The P_R is estimated from the sample of all patients irrespective of treatment group who were a probable cure at D56. P_R is equal to the number of relapses between D56 and D210 divided by the number of probable cure at D56 who attend D210 visit.

	The imputed probability of success (IP_S) will be set at $1 - 2P_R$ and the number of imputed success in a given treatment group will be equal to the number of unconfirmed status multiplied by $1 - 2P_R$. The relapse rate (P_R) is multiplied by two to impose a penalty when the status at D210 is unknown. Considering that all 3 study arms in this non-inferiority trial are expected to have similar true relapse rate after day 56 efficacy at D210 (similar proportion of relapses), the imputation method will consider pooled P_R as the best estimation of failure rate. If the most efficient combination has a smaller relapse rate, then the measure of the effect size will be in disfavor of this combination. <u>In addition, the following sensitivity analyses will be performed:</u> 1) Replacement of the primary imputation approach by the conservative approach (LTFU = failure). 2) Replacement of the primary imputation approach by the optimistic approach (LTFU = success). 3) Replacement of the primary imputation approach by the realistic approach (LTFU have the same rate of relapse as completers). 4) Replacement of the mITT by the per-protocol set of patients. 5) Replacement of the mITT by the set of completers. This analysis avoids any imputation but is based on a subset of patients. The result should be close to the third sensitivity analysis if there are not many patients with unconfirmed status.

Table 1 - Schedule of events

Protocol Activities and Forms to Be Completed	Screening	Treatment period (day) +/- 1d except for D28						Follow up visits	
	D-7 to D0	D1	D3	D7	D14	D28 (EOT) +1d		D56 +/- 7d	D210 +/- 14d
Consent form & consent for HIV test	X								
Demographic data and medical history	X								
Clinical assessment	X	X	X	X	X	X		X	X
Nutritional status	X								
Audiometric test	X				X	X			X
HIV test	X								X[1]
Pregnancy test[2]	X					X		X	X
Spleen/bone marrow/lymph node aspiration, parasitology assessment (microscopy)	X					X		X[6]	X[6]
Hematology (hemoglobin, WBC with differential, platelets)	X		X	X	X	X		X	X
Biochemistry analysis (AST/ALT, bilirubin, creatinine, albumin[3])	X		X	X	X	X			
ECG[4]	X			X	X	X			
Assessment for PKDL	X					X		X	X
Blood sample for miltefosine PK[5]						X		X	
Tissue (bone marrow/spleen/lymph node) and blood sample for PD (qPCR)[5]	X					X		X[6]	X[6]
MF treatment		*MF allometric dose BID for 14 or 28 days*							
PM treatment		*PM QD for 14 days*							
SSG/PM treatment		*SSG/PM QD for 17 days*							
Safety assessment	SAEs/non-serious study related AEs	---------- SAEs and AEs monitoring during the study ----------							

[1] HIV test at D210 will be done only for patients in Gondar.
[2] Only for woman of child-bearing potential.
[3] Albumin will be assessed as a marker of VL evolution only at screening, day 28 and day 210.
[4] ECG will be performed at baseline and at D14 in all arms, and in addition at D7 and D28 in the reference arm SSG/PM.
[5]These assessments will apply to all patients enrolled in the trial, except the ones included in the intensive cohort (see section 5.1 Schedule of PK/PD events – Intensive cohort). PK MF sample will be collected prior to the morning dose at D28. D56 sample will be collected in the morning.
[6]Parasitology and collection of tissue and blood samples for qPCR will be done during the follow-up period after D28 (at D56 and D210) only <u>if clinically indicated</u> (i.e. anytime if suspect of relapse, with reappearance of symptoms and signs of VL).
Total volume of blood to be collected for patient not included in the intensive PK/PD cohort:
Assumptions: Ht= 1mL EDTA, BQ= 2mL dried tube, MF PK= 2mL EDTA, PD= 1mL EDTA. Volume of blood to be collected will be minimized as much as possible, according to the volume of tubes available in the market. The maximum volume to be collected at each study visit will be 6mL.

Table 2 - Schedule of PK/PD events - Intensive cohort

40 patients from Kenya and Sudan in each arm 1 and 2 (20 paediatric (≥7 to ≤12 yrs) patients and 20 adults) will be included in the intensive cohort and will have a separate schedule of PK and PD events as described below.

Protocol Activities and Forms to Be Completed	Screening	Treatment period (day) +/- 1d except for D28							Follow up visits	
	D-7 to D0	D1	D3	D7	D14	D21[1]	D28 (EOT) +1d		D56 +/- 7d	D210 +/- 14d
Sampling Time-points:										
Blood sample for paromomycin PK[2]	X	X			X					
Blood sample for miltefosine PK[3]	X	X		X	X	X	X		X	
Blood sample for PD (qPCR)	X		X	X	X		X		X[4]	X[4]
Sampling Volumes:										
Total EDTA tube blood volume (mL)	**4**	**12**	**2**	**4**	**12**	**2**	**4**		**4**	**2**
Blood volume for hematology (mL)	1		1	1	1		1		1	1
Blood volume for PK plasma (mL)	2	12		2	10	2	2		2	
Blood volume for PD qRT-PCR (mL)	1		1	1	1		1		1	1
Total dried tube blood volume (for biochemistry)	**2**		**2**	**2**	**2**		**2**			
TOTAL BLOOD VOLUME (mL)[5]	**6**	**12**	**4**	**6**	**14**	**2**	**6**		**4**	**2**

[1] D21 visit will only be applicable to the subset of patients allocated to intensive PK sampling in arm 2.
[2] PK PM sample at D1 and D14 (arm 1 and 2):
In order to minimize blood volume collected, a subset of patients will be randomly allocated to one sparse PK sampling schedule:
- D1: either 1, 2, 4, 24h or 1, 2, 8, 24h
- D14: either 0, 1, 2, 4, 24h or 0, 1, 2, 8, 24h

[3] PK Miltefosine sample at D1 and D14 will be collected at the same time points as PK PM (according to the sparse sampling scheme the patient is allocated to). 8h sample will be collected always before the 2nd daily dose. Additional PK samples will be collected prior to the morning dose at D7, D21 (arm 2 only) and D28. D56 sample will be collected in the morning.
[4] Blood samples for qPCR will be collected during the follow-up period (at D56 and D210) only if clinically indicated (i.e. anytime if suspect of relapse, with reappearance of symptoms and signs of VL).
[5]Volume of blood to be collected will be minimized as much as possible, according to the volume of tubes available in the market.
The maximum volume to be collected at a study visit will be 14 mL on day 14.

Planning Information

Study Timelines

Final protocol available	February 2017
Study treatment supply available	May 2017
FSFV	Q3 2017
Duration of recruitment period	14 to 17 months (Q3 2017 to Q1 2019)
Duration of follow-up period	6 months
LSLV	Q3 2019
Interim analysis	N/A
Final study report	Q1 2020

STUDY SCOPE

Target countries	Kenya, Uganda, Sudan, Ethiopia
Enrollment target	576 subjects
Number of sites	6
Number of subjects per site	There will not be a minimum requirement of number of patients enrolled per site. Once EC/regulatory approvals are obtained in the country, recruitment shall start, and other sites to be added as approvals are obtained.
DSMB involvement	A Data Safety Monitoring Board will be appointed for this study
Partners involvement	KEMRI, Kenya Makerere University, Uganda Institute of Endemic Diseases, University of Khartoum, Sudan University of Gondar, Ethiopia Research Foundation of the Netherlands Cancer Institute (NLADF), the Netherlands

Study Treatments Supply

Study medications	Product: Miltefosine (Impavido®) Commercial source: Knight Therapeutics Inc. / Paladin Inc. Presentation: 10mg and 50mg capsules in packs of 56 capsules sealed in 8 aluminium blister stripes, each containing 7 capsules. Product: Paromomycin Commercial source: Gland Pharma Presentation: Each 2 ml ampoule contains approximately 1g PM sulphate equivalent to 750 mg PM base/2ml (375 mg/ml). Product: Sodium Stibogluconate Commercial source: Albert David Ltd. Presentation: Each vial contains SSG 100 mg/ml 33% 30 ml inj. (10% antimony).
Labeling instructions	MF, PM and SSG packs to be used in the clinical trial will have trial specific labels with the following statement *"For Clinical Trial use Only"* and including protocol code, Sponsor and PI contact details as well as direction for use.