DNDi
Drugs for Neglected Diseases *initiative*

CLINICAL TRIAL PROTOCOL

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

Short title	MF/PM Phase III
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
Clinical Trial Protocol Number	DNDi-MILT/PM-01-VL

Sponsor	DND*i* (Drugs for Neglected Diseases *initiative*), Chemin Louis Dunant, 15, 1202 Geneva, Switzerland Phone: +41 22 906 9230 Acting through its DNDi Africa office, Tetezi Towers, 3rd Floor, George Padmore Road, Kilimani, P.O. Box 21936 - 00505, Nairobi, Kenya Phone: +254 20 3995 000 Sponsor's representative: Dr Monique Wasunna, Director, DNDi Africa Regional Office, Nairobi, Kenya

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Current Clinical Trial Protocol Version / Date	Draft version 5.0, 13 Mar 2020
Previous Clinical Trial Protocol Version / Date	Final version 1.0, 20 Feb 2017 Final version 2.0, 5 Sep 2017 Final version 3.0, 4 May 2018 Final version 4.0, dated 22 Jul 2019

CLINICAL TRIAL PROTOCOL SIGNATURE PAGE

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I have read this protocol and agree that it contains all necessary details for carrying out this trial. I will conduct the trial as outlined herein and will complete the trial within the time designated.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this trial. I will discuss this material with them to ensure they are fully informed regarding the drug and the conduct of the trial.

I will use only the informed consent form approved by the sponsor or its representative and will fulfill all responsibilities for submitting pertinent information to the Institutional Review Board/Independent Ethics Committee (IRB/IEC) responsible for this trial if required by national law.

I agree that the sponsor or its representatives shall have access to any source documents from which case report form information may have been generated.

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ABBREVIATIONS – GLOSSARY OF TERMS

AE	Adverse event
ALB	Albumin
ALT	Alanine aminotransferase (SGPT)
AR	Adverse Reaction
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve
BID (b.i.d.)	Twice a day
BMI	Body Mass Index
CI	Confidence Interval
CIOMS	Council for International Organizations of Medical Sciences
Cmax	Maximum concentration
CRE	Creatinine
CRF	Case report form
CTCAE	Common Terminology Criteria for Adverse Events
DNDi	Drugs for neglected diseases initiative
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
EMA	European Medicines Agency
EOT	End of treatment
FAS	Full Analysis Set
FDA	Food and Drug Administration
GCP	Good clinical practice
GMP	Good manufacturing practice
HED	Human Equivalent Dose
HIV	Human immunodeficiency virus
IA	Interim Analysis
ICH	International Conferences on Harmonization
IEC	Independent ethics committee
IM	Intramuscularly
IMP	Investigational Medicinal Product
ITT	Intention to treat
IV	Intravenous
LC-MS/MS	Liquid chromatography mass spectrometry
LEAP	Leishmaniasis East Africa Platform
MedDRA	Medical Dictionary for Regulatory Activities
MF	Miltefosine
MoH	Ministry of Health
MUAC	Mid Upper Arm Circumference
MSF	Medecins Sans Frontières (Doctors without Borders)
NCE(s)	New Chemical Entity(ies)
PCR	Polymerase chain reaction
PD	Pharmacodynamic
PI	Principal investigator
PK	Pharmacokinetics
PKDL	Post Kala azar Dermal Leishmaniasis
PM	Paromomycin
PP	Per Protocol

PT	Prothrombin Time
QD (q.d.)	Daily
RDT	Rapid Diagnostic Test
SAE	Serious adverse event
SOP	Standard Operating Procedure
SSG	Sodium Stibogluconate
TBIL	Total Bilirubin
TEAE	Treatment emergent adverse event
ULN	Upper limit of normal
VCT	Voluntary counselling and testing programme
VL	Visceral Leishmaniasis
WBC	White blood cell
WHO	World Health Organization

PROTOCOL SYNOPSIS

Protocol title	An Open Label, Phase III, Randomized Controlled, Multicentre Non-Inferiority Trial to Compare Efficacy and Safety of Miltefosine and Paromomycin with Sodium Stibogluconate and Paromomycin Combination for Treatment of Primary Visceral Leishmaniasis (VL) Patients in Eastern Africa
Short title	MF/PM Phase III
Clinical Trial Protocol Number	DNDi-MILT/PM-01-VL
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PROTOCOL SUMMARY

Current WHO-recommended treatment for Visceral Leishmaniasis (VL) in Eastern Africa is a combination of Sodium Stibogluconate (SSG) and paromomycin (PM) administered for 17 days, with an efficacy of 91%. This treatment is far from optimal as it requires 17 days of two separate painful injections, necessitating patients to be hospitalized during the whole treatment period. In addition, the antimonial SSG exhibits life-threatening toxicities such as cardiotoxicity, hepatotoxicity and pancreatitis. Therefore, there is an urgent need to explore alternatives that are efficacious, safe, ideally of short duration, affordable and suitable to be used in remote areas where VL occurs.

Until new chemical entities are developped, 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.

Miltefosine (MF) is the only oral drug available for VL treatment. It has been extensively used in Asia for VL treatment as monotherapy for 28 days, with satisfactory cure rates (>90%). However, MF as monotherapy showed lower efficacy in Eastern Africa (72%, 95%CI: 60–85%) than in Asia. PK data indicated under-exposure and higher relapse rates in children compared to adults with the conventional linear 2.5 mg/kg/day MF dose. To overcome this under-dosage in children, an allometrically scaled dosing regimen has been developed. This allometric dosage was assessed for paediatric VL in the LEAP 0714 trial in Kenya and Uganda. The 28 days regimen of MF allometric dose showed 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 the efficacy level could be increased in children treated with the allometric dose, reaching similar efficacy observed in adults (i.e. 86.2%).

PM has been well studied in Eastern Africa during the development of the combination SSG-PM. PM monotherapy of 20mg/kg/d IM for 21 days showed an overall efficacy at 6 months of 84.3%. 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.

Replacing the toxic SSG with oral MF can bring better safety and a more field-adapted, patient-friendly treatment.

Originally, the study was planned to assess the safety and efficacy of two combination regimen of PM and MF (arm 1: PM/MF for 14 days and arm 2: PM for 14 days and MF for 28 days) as compared to the standard of care SSG-PM for the treatment of primary VL patients in Eastern Africa. Due to unforeseen challenges in recruitment, the study design has been amended to prematurely discontinue recruitment in one investigational arm in order to complete the trial in an acceptable timeframe and ensure the achievement of the objectives.

Following DSMB review of the available data (meeting held on 7 May 2019), including 143 study participants enrolled, among which 135 had completed treatment and Day 28 follow up assessment, and 66 had completed their day 210 follow-up visit, two main changes were approved: first, the revision of the exclusion criteria in order to include a more VL representative population, and second, to discontinue recruitment in the investigational arm 2.

These modifications in agreement with the DSMB will ensure retention of the investigational arm 1, on the basis of 1) shorter treatment duration than current standard of care, 2) it can be administered within the hospitalization period, 3) it is a cheaper option for the control programs, and therefore from a public health perspective will be a potentially more favorable option for patients.

In this revised protocol version, the analysis remains as planned, but comparison will be performed only between one investigational arm (arm 1 MF/PM 14 days) and the standard of care (SSG-PM). Data from patients randomized in arm 2 (PM 14 days/MF 28 days), which recruitment is now stopped, will be summarized in a descriptive analysis, but not included in the formal comparative efficacy analysis.

The current study aims to determine the safety and efficacy of one combination regimen of PM and MF as compared to SSG-PM for the treatment of primary VL patients in Eastern Africa. This is an open label, Phase III, randomized controlled, parallel arm multicentre non-inferiority clinical trial. The investigational arms are:

- Arm 1: Paromomycin 20 mg/kg/d IM for 14 days combined with oral miltefosine allometric dosing BID for 14 days
- Arm 2: Paromomycin 20 mg/kg/d IM for 14 days combined with oral miltefosine allometric dosing BID for 28 days **(recruitment is now discontinued in this arm)**

The control arm is the current standard treatment for VL in East Africa:

- Arm 3: Sodium Stibogluconate 20 mg/kg/day IM/IV combined with Paromomycin 15 mg/kg/day IM for 17 days

MF allometric dosing will be calculated according to patient's weight, height and sex. 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, patients weighing ≥ 30 to < 45 kg will receive

100 mg/day and patients ≥ 45 kg will receive 150 mg/day.

Subjects are hospitalized for 14 days of PM and MF treatment in both arm 1 and arm 2. MF treatment starts at the same time as PM treatment. At discharge (on day 15), patients allocated to arm 2 used to be instructed to continue MF treatment on an out-patient basis until completion of the 28 days treatment. Subjects were receiving clear instructions as well as a daily diary to guide them in their treatment schedule at home.

SSG-PM combination therapy will be administered for 17 days according to routine VL treatment guidelines and patients will remain hospitalized for the entire duration of the treatment. Patients in all arms will come back for the end-of-treatment assessments visit at day 28.

The target population will be primary VL patients from 4 to 50 years. It is important to include a paediatric population which is particularly vulnerable and is a major factor in the East African disease burden. The trial will be run in the VL endemic countries at 5 LEAP sites: Kacheliba in Kenya, Amudat in Uganda, Doka and Um El Kher in Sudan and Gondar in Ethiopia, as well as 2 MSF sites: Tabarakallah in Sudan and Abdurafi in Ethiopia.

Revised enrollment target in protocol version 4.0 dated 22 July 2019 is 170 in each study arm 1 and 3. The total number of patients included in the trial will be approximately 420, taking into account the number of patients randomized to treatment arm 2 before discontinuation of this arm (approximately 80 patients). Each patient's participation in the study will be for approximately 7 months. This will consist of baseline assessments, treatment period (14 or 17 days) and 6 months follow-up. Recruitment for the entire trial is expected to take approximately 32 months assuming that 15 to 30 % 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 39 months. Taking into account the analysis and reporting period, the study shall last at most 45 months.

Primary efficacy endpoint will be the cure at day 210, based on clinical examination (absence of clinical signs and symptoms of VL and no requirement for rescue treatment during the trial).

Safety assessments will be done through routine monitoring of adverse events. A characterization of the nature and frequency of SAEs, AEs that lead to treatment discontinuation and overall frequency and severity of AEs from start of the treatment until 6 months follow-up will be made.

Pharmacokinetics (PK) profile of PM and MF will be described and parasite clearance in each arm as indicated by direct microscopy and quantitative polymerase chain reaction (qPCR) will be evaluated as pharmacodynamics (PD) markers of cure. The relationship between PK and PD measurements will be further assessed through drug exposure-response modelling.

Finally, compliance to MF treatment in an outpatient setting will also be described. If proven non-inferior to SSG-PM, the combination of PM and MF is expected to improve significantly treatment safety and to reduce pain, while ensuring low cost and suitability to be used in remote areas. It will provide the East African region with the first non-antimony-based effective treatment for VL.

1. Background and Study 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: Brazil, Ethiopia, India, Sudan, Somalia and South Sudan (Alvar et al., 2012). In Eastern Africa, VL is caused by *Leishmania donovani*, affecting mainly children (Harhay et al., 2011).

Eastern Africa is expected to become the VL region with the highest burden worldwide 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 (WHO, 2010). 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 (Musa et al., 2012; Hailu et al., 2010). This new treatment is an improvement over the 30-day SSG monotherapy, but a few important drawbacks and 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® in Eastern Africa.

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.

In Asia, a 10-days combination of PM with MF has been very successful with a definitive cure rate at 6 months of 98.7% (95% CI: 95.1-99.8%) (Sundar et al., 2011). These results were confirmed in an implementation study in India where the MF/PM combination showed 96.9% (95%CI: 95.0-98.2%) efficacy at the end of 6 months follow-up, which was similar for all age groups. The regimen needs to be adapted to the African disease context which is expected to require longer duration and higher

doses to achieve satisfactory efficacy, as described below based on the experience of miltefosine and paromomycin treatment in East Africa.

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 registered in India for VL indication in 2002, and it has been extensively used in Asia for VL treatment as monotherapy for 28 days. Treatment of up to 12 weeks is recommended for Post Kala azar Dermal Leishmaniasis (PKDL) in Asia. It is well tolerated, the main side effects are vomiting and nausea, and transient increases in liver enzymes and creatinine. The 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 (28 days) and after treatment for at least 5 months. Furthermore, in the US Product Information (Paladin Therapeutics, 2014) 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) (FDA Advisory Committee & Book, 2013). 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, Omollo et al., 2011; Wasunna et al., 2016). 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 adolescents/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, Huitema, Beijnen, & De Vries, 2012; Ostyn et al., 2014). MF has a long half-life (7 days), and keeps accumulating over the treatment duration to eventually reach 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 (Dorlo et al., 2012). 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 MF 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 (T. P. C. Dorlo et al., 2014). 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 (Paromomycin Package Insert, 2016).

PM has been well studied in Eastern Africa during the development of the combination SSG-PM. The initial PM monotherapy arm in the LEAP 0104 trial 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%) (Hailu et al., 2010). 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); the efficacy at the 6-months follow-up was 81% (17/21, 95%CI 58-95%) and 80% (16/20, 95%CI 56-94%) respectively (Musa et al., 2010). 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%) (Musa et al., 2012). 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 MF and PM treatment regimen is non-inferior to the SSG-PM combination currently used as first line treatment in Eastern African VL patients. If proven efficacious (non-inferior) and safe, 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.

Originally, the study protocol had a trial design including three arms: two investigational arms with MF and PM and the reference treatment arm SSG-PM for 17-day course treatment. The strategy was 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) aiming to reduce as much as possible the hospitalization period, while achieving appropriate exposure and satisfactory efficacy levels.

The trial started with both test arms: MF/PM for 14 days and MF for 28 days/PM for 14 days. During the first year of recruitment in the trial, the number of VL cases in the

region dropped by 20% as compared to the preceding 4 years before commencing the trial. In addition, only 4 out of 7 sites have been actively recruiting patients for various reasons, including political instability in the countries, affecting local operations. Finally, the enrolment rate (% of 'VL patients recruited in the trial / VL confirmed cases') has been lower than anticipated and variable across sites, with an overall enrolment rate of only 16% as compared to the 30% planned. Therefore, the study with its original design could not be conducted in a reasonable timeframe and important changes have been considered in order reach the study objectives.

In order to mitigate the low enrollment, the protocol was amended to v3.0 dated 4 May 2018 to include 2 MSF sites (Tabarakallah in Sudan and Abdurafi in Ethiopia). The sites were expected to compensate for the low patient numbers overall. In addition, because of the high proportion of patients in Ethiopia excluded due to severe malnutrition (30-40%), an Ethiopia-specific amendment was implemented to modify the indicator for severe malnutrition from body mass index (BMI) to mid-upper arm circumference (MUAC) to allow for a better representativeness of the Ethiopian population. Finally, active case search and referral networks were initiated at some of the sites. Nevertheless, these measures taken to improve recruitment have not resulted in a significant boost of the enrolment and further actions needed to be taken.

New oral promising NCEs are expected to be at the clinical stage of development for VL patients by 2021 and the plan is to complete this trial before then so that results can be of benefit to the VL patients. Moreover, if new trial(s) with NCEs start before the end of this study, the recruitment of patients will compete with these NCEs studies and will become even more challenging to develop new treatments for visceral leishmaniasis in Eastern Africa region.

Following DSMB review of the available data (meeting held on 7 May 2019), two main changes to the protocol were agreed to achieve completion of the trial in an acceptable timeframe and ensure the achievement of the objectives of the trial. First, to revise the exclusion criteria in order to include a more VL representative population, and second, to discontinue recruitment in the investigational arm 2.

Revision of the exclusion criteria. The reasons for exclusions leading to a low enrollment rate were analyzed. It was noted that across the sites, 15 to 20% of patients were excluded because of laboratory abnormalities. In most cases, the baseline laboratory abnormality was not considered clinically significant by the investigator to indicate AmBisome therapy and a majority of these patients were therefore treated with SSG-PM with a good therapeutic response. For this reason, individual laboratory parameters in the exclusion criteria were replaced by investigator judgement on the need of AmBisome, based on the severity of the clinical manifestations of the disease (such as jaundice, bleeding and oedema) and laboratory parameters.

Discontinue recrutiment in investigational arm 2 (PM 14d / MF 28 days). Following review of the preliminary available data by the DSMB, including 143 study participants enrolled, among which 135 had completed treatment and Day 28 assessment, and 66 of them had completed their day 210 follow-up visit, it was decided in agreement with the DSMB to ensure retention of the investigational arm 1, on the basis of 1) shorter treatment duration than current standard of care, 2) it can be administered within the hospitalisation period, 3) it is a cheaper option for the control programs, and therefore from a public health perspective will be a potentially more favorable option for patients.

In this revised protocol version, the analysis remains as planned, but comparison will be performed only between one investigational arm (arm 1 MF/PM 14 days) and the standard of care (SSG-PM). Data from patients randomized in arm 2 (PM 14 days/MF 28 days), which recruitment is now stopped, will be summarized in a descriptive analysis, but will not be included in the comparative hypothesis testing.

The total dose of PM will be 280 mg, similar to the total dose given in the SSG-PM combination therapy (255 mg). For MF, PK data indicated that with 14 days allometric MF treatment, 91% of patients will at least reach EC90 in plasma, which would represent appropriate exposure.

If demonstrated non-inferior to SSG-PM, the 14-day MF-PM regimen will be preferable to be taken forward for future standard of care or alternative treatment to SSG-PM. Data will be presented to Ministry of Health (MoH) and regulatory authorities in the region as evidence to support policy change for the national control programs.

2. Study Objectives and Endpoints

2.1 Objectives

2.1.1. Primary Objective

- To compare the efficacy of a 14-day combination regimen of MF and PM with the standard 17-day course of SSG-PM for the treatment of primary VL patients in Eastern Africa

2.1.2. Secondary Objectives

- To assess 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 efficacy of the PM (14 days) and MF (28 days) investigational arm
- 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 describe compliance to MF treatment in an outpatient setting

2.2. Study Endpoints

2.2.1. 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 (i.e. no relapse or initial treatment failure).

2.2.2. Secondary Endpoint(s)

- **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 treatment.

- **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, during treatment and until the last point of follow-up, and for PM 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 described through patients' hospital records history, drug accountability and PK measurements.

3. Study design and study design rationale

3.1. Study design

This is an open label, Phase III, randomized, controlled, parallel arm multicentre non-inferiority clinical trial to compare the efficacy of a combination regimen of MF and PM with SSG-PM for the treatment of primary adult and children VL patients in Eastern Africa.

In addition, safety of two combination regimens of PM (14 days) and MF (14 or 28 days) will be assessed, and the efficacy of the PM (14 days) and MF (28 days) investigational arm 2 will be described.

The study will be conducted at 5 LEAP sites and 2 MSF sites: Kacheliba in Kenya,

Amudat in Uganda, Doka, Um El Kher and Tabarakallah (MSF) in Sudan and Gondar and Abdurafi (MSF) in Ethiopia.

The 2 treatment regimens to be tested were as below but with patient's recruitment in arm 2 now discontinued:

- **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 **(recruitment is now discontinued in this arm but patients' data available will be analyzed and results described)**

The reference arm is the current standard treatment for VL:

- **Arm 3**: Sodium Stibogluconate 20 mg/kg/day IM/IV 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 < 45 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 (Kimutai et al., 2017). MF and PM 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 BID for 14 days in arm 1 and for 28 days in arm 2.

Patients are hospitalized for 14 days of PM and MF treatment for both arm 1 and arm 2. MF treatment starts at the same time as PM treatment and for arm 2 it used to 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 were discharged after 14 days in hospital with clear instructions on how to continue MF treatment, and to return for the Day 28 visit. Compliance during the 14 days treatment outside the hospital was assessed by collecting the empty blisters pack at the day 28 visit and checking for any unused drug. A daily diary was provided to these patients in order to guide them in their treatment schedule at home. Any episode of vomiting reported by the patient was captured by the clinician at day 28 visit. In case of multiple episodes of vomiting or any other adverse events, patients were 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. Patients in all arms will come back for the end-of-treatment assessments visit at day 28.

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: originally, 40 in each MF/PM combination arms, with selection of 20 pediatric (≥7 to ≤12 yrs) patients and 20 adolescent/adult patients (>12 to ≤50 years). Because of premature discontinuation of arm 2, the number of patients enrolled in this arm in the PK/PD intensive cohort will probably not reach 40, however samples collected will be analysed. 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 section 5.1 Schedule of PK/PD events – Intensive cohort, as well as section 8.4 Pharmacokinetics and Pharmacodynamics Assessments).

3.2. Study duration and duration of subject participation

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 approximately 32 months assuming that 15 to 30% 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 39 months. Taking into account the analysis and reporting period, the study shall last at most 45 months.

3.3. Rationale of study design

The strategy to combine one parenteral drug for a short period (PM 20 mg/Kg/d IM for 14 days) with an oral drug for the same or longer duration (MF allometric dosing for 14 or 28 days) was to reduce as much as possible the hospitalization period and to allow the treatment to achieve appropriate exposure and satisfactory efficacy levels for the treatment of primary VL patients. In addition, replacing the toxic SSG with oral MF can bring better safety and a more field-adapted, patient-friendly treatment. NCE candidates won't be fully developed for VL treatment before 2027. Therefore, currently available compounds should be assessed to improve on current recommended treatment and avoid adverse drug reactions observed with SSG.

The primary endpoint of this protocol is cure at 6 months follow up defined as absence of signs and symptoms of VL (and no rescue treatment), aiming to assess efficacy of the regimen of MF combined with PM as compared to the routine VL treatment SSG-PM. The long term follow-up of 6 months is necessary in VL trials due to risk of relapse after a negative test of cure at the end of treatment.

This phase III trial should provide the evidence to determine if the investigational study arm is non-inferior to SSG-PM. Patient tolerability will improve with short course combination regimens that are better tolerated and more patient-friendly.

Safety assessments will include clinical, haematological and biochemistry evaluations at regular time points (day 0, 3, 7, 14, 28, 56 and 210) to ensure safety is adequately

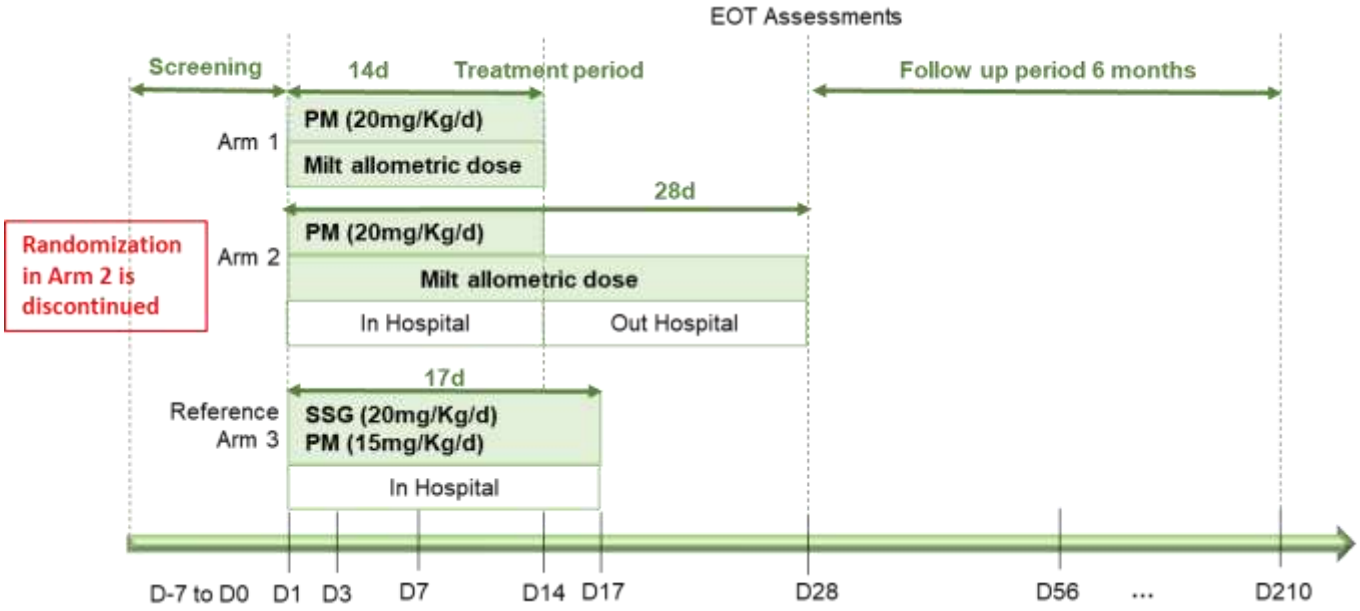
assessed and treatment failures can be caught.

Samples size estimation is based on the power and precision to estimate the efficacy of definitive cure at 6 months (D210) which is the primary endpoint and main parameter of interest. Taking into consideration the data available on PM monotherapy in East Africa, the results of LEAP 0208 on miltefosine efficacy (Wasunna et al., 2016) and latest efficacy results of MF allometric dose in children in Eastern Africa, the expected efficacy for the combination of PM with MF at the end of 6 months follow-up will be defined as 93%. The margin defined as acceptable for concluding the non-inferiority of the tested treatments compared to the reference arm (SSG-PM, 91% expected efficacy) will be 7%, considering the advantages of MF/PM combination in terms of safety, shorter hospitalization and more patient-friendly.

An interim analysis (IA) will not be performed in this trial. Unfortunately, there is no early marker of cure for VL that would allow an interim analysis using data from end of treatment to predict the outcome at the 6 months follow-up.

Pharmacodynamic parameters of repeated qPCR measurements in blood and in tissue (baseline and EOT), and clinical outcome up to D210 will be assessed to allow for a simultaneous population PK-PD analysis to investigate in detail the drug exposure-treatment response relationship.

Figure 1- Overall study design



4. Selection of Subjects

170 patients will be enrolled in each study arm 1 and 3. The total number of patients included in the trial will be approximately 420, taking into account the number of patients randomized to treatment arm 2 before discontinuation of this arm (approximately 80 patients). Patients will be primary VL cases with age range between 4 and 50 years of age.

In order to meet recruitment target, 5 LEAP sites and 2 MSF sites are included in the trial: Kacheliba in Kenya; Amudat in Uganda; Doka, Um El Kher and Tabarakallah (MSF) in Sudan; and Gondar and Abdurafi (MSF) in Ethiopia.

Sites will be initiated as soon as the required EC/regulatory approvals have been obtained in their respective country.

Approximately 150 VL patients are treated routinely per month in the 7 sites. Based on historical data from previous trials, whereby it is estimated that 15% of patients will not be in the age range of 4-50 years, 20% will be excluded based on lab and/or nutrition criteria, 15% will be female patients not willing or not able to comply with contraception and an additional 30% may not be enrolled for other criterion, the enrollment rate for this trial will thus be approximately 20% of the total number of patients diagnosed with VL. Therefore, the recruitment capacity expected is 20 patients per month when all trial sites are active. Considering a mean approval process of 6-12 months in the countries, the delay in the construction of Umelkher site and the fact that MSF sites have joined the trial at a later stage, the target enrollment of 170 patients per arm is planned to take approximately 32 months.

There will not be a minimum requirement of number of patients enrolled per site; sites are expected to recruit competitively until the total sample size is achieved.

Enrollment will be boosted by increasing patient outreach through local strategies such as active case search.

The following eligibility criteria were designed to select subjects for whom the protocol treatment is considered appropriate. All relevant medical and non-medical conditions should be taken into consideration when deciding whether this protocol is suitable for a particular subject. Eligibility criteria may not be waived by the investigator. Any questions regarding a subject's eligibility should be discussed with the DNDi Medical Coordinator prior to subject's enrollment.

4.1. Inclusion criteria

Subjects must meet **all** of the following inclusion criteria to be eligible for enrolment into the study:

- 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

4.2. Exclusion criteria

The presence of any of the following will exclude a subject from study enrolment:

- Patients who are relapse cases

- Patients with Para-Kala azar dermal leishmaniasis grade 3
- 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 patients 5-18 years: BMI-for-age WHO reference curves by sex, z score < -3; for adults >18 years: BMI < 16)*
- Patients with positive HIV diagnosis
- Patients with previous history of hypersensitivity reaction or known drug class allergy 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, schistosomiasis or any other serious underlying disease (e.g. cardiac, renal, hepatic) or chronic condition 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 (see section 15.2)
- Patients with haemoglobin < 5g/dl
- Patient with signs of severe VL according to Investigator's judgement, requiring an indication for AmBisome therapy based on the clinical manifestations (such as jaundice, bleeding, edema) and clinically significant abnormalities in the following laboratory parameters: haemoglobin, WBC, platelets, liver enzymes (ALT and AST), total bilirubin and creatinine
- Patients with pre-existing hearing loss based on audiometry at baseline
- Patients who cannot comply with the planned scheduled visits and procedures of the study protocol

** Note: for Ethiopia only: Patients with severe malnutrition (for patients 4-18 years: MUAC cut-off based on MUAC-for-height reference table; for patients > 18 years: MUAC < 170 mm)*

5. 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[5]	X[5]
Hematology (hemoglobin, WBC with differential, platelets)	X		X	X	X	X		X	X
Biochemistry analysis (AST/ALT, bilirubin, creatinine)	X		X	X	X	X			
Biochemistry analysis (Albumin)	X					X			X
ECG[3]	X			X	X	X			
Assessment for PKDL	X					X		X	X
Blood sample for miltefosine PK[4]					X	X		X	
Tissue (bone marrow/spleen/lymph node) and blood sample for PD (qPCR)	X					X		X[5]	X[5]
Treatment arm 1		*PM QD/MF allometric dose BID for 14 days*							
Treatment arm 2 – **Discontinued**		*PM QD for 14 days /MF allometric dose BID for 28 days*							
Treatment arm 3		*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 and Abdurafi.

[2] Only for woman of child-bearing potential.

[3] 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.

[4] MF PK assessments at D14 will apply to all patients enrolled in arm 1, while D28 and D56 samples will apply to all patients enrolled in arm 1 and 2. MF PK sample will be collected prior to the morning dose at D14 and D28 (arm 2). D28 (arm 1) and D56 samples will be collected in the morning.

[5] 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 if clinically indicated (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.

General note: PK and PD assessments in this schedule of events do not apply to patients included in the intensive cohort (see section 5.1 Schedule of PK/PD events – Intensive cohort).

5.1 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 adolescent/adult patients >12 to ≤50 yrs) will be included in the intensive cohort and will have a separate schedule of PK and PD events as described below. Because of discontinuation of arm 2 in protocol v4.0 dated 22 July 2019, the number of patients in arm 2 enrolled in the PK/PD intensive cohort is expected to be probably less than 40.

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**			**2**
TOTAL BLOOD VOLUME (mL) [5]	**6**	**12**	**4**	**6**	**14**	**2**	**6**		**4**	**4**

[1] D21 visit will only be applicable to the subset of patients allocated to intensive PK sampling in arm 2 (collection of this sample is not applicable anymore in this protocol version).
[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. PK samples will be collected prior to the morning dose at D7 and D28 (arm 2). D28 (arm 1) and D56 samples 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.

6. Enrolment procedures

The study will be conducted in the LEAP and MSF sites. These sites are located in endemic areas of VL and are reference treatment centers in the regions, where routinely VL patients are diagnosed and treated.

Patients may come to the treatment center passively or through referral from health professionals. Referral can be done on the basis of clinical signs and symptoms and/or rapid diagnostic test. In some of the sites, active case detection is performed by mobile teams visiting regularly highly endemic communities. This procedure will be boosted during the inclusion period of the clinical trial.

Subjects between the ages of 4 and 50 years who fulfill the inclusion / exclusion criteria, and from whom informed consent has been obtained (see 15.1 Informed Consent process) will be enrolled in the study. Patients who have entered screening assessments for this trial will be documented in a Patient Screening/Enrolment Log. Each eligible patient will be assigned a patient identification number by the investigator in the site, sequentially, which will be recorded in the Patient Screening/Enrolment Log.

A note describing the ICF signature and enrolment in the trial will also be made in the patient's file.

Patients were originally randomized centrally to treatment in a 1:1:1 allocation ratio using varying block sizes of 6, 9 or 12 subjects in random order. Following discontinuation of arm 2, a 1:1 allocation ratio is now used with varying block sizes of 4, 6 or 8 subjects. Randomization codes will be prepared by the DNDi Data Center in Nairobi and will be accessible through an online system. If the principal investigator has no internet connection available, randomization codes will also be provided by phone.

Treatment should be started within 7 days after the screening assessment. Otherwise compliance with eligibility criteria has to be reassessed (blood tests).

Enrolment status of subjects at each site will be informed weekly by the sites to the study monitor and updated by data centre. Recruitment will stop once 170 subjects have been enrolled in each trial’s arm 1 and 3.

7. Study treatments

7.1. DNDi study treatment

Name: **Miltefosine (Impavido®)**
Class: Phosphocholine analogue.
Mechanism of action: interferes with the synthesis and metabolism of phospholipids. It may also interfere with the parasite’s membrane signal transduction, and glycosylphosphatidylinositol anchor biosynthesis.
Commercial source: Paladin labs and Knight Therapeutics Inc.
Product appearance: comes as 10mg and 50mg capsules in pack of 56 capsules sealed in 8 aluminum blister stripes, each containing 7 capsules.
Administration: oral treatment
For MF, there are no known interactions with other commonly used medications, though this cannot be excluded.

Name: **Paromomycin**
Class: Aminoglycoside
Mechanism of action: inhibits protein synthesis by binding to 16S ribosomal RNA.
Commercial source: Gland Pharma, India; distributed by IDA Foundation.
Product appearance: Each box of paromomycin contains a pack of 10 amber-colored glass ampoules containing 750 mg of paromomycin base in 2 ml (375 mg/ml) equivalent to 1 g of paromomycin sulphate in 2 ml (500 mg/ml).
The 20 mg/kg sulfate is equivalent to 15 mg/kg of base.
Administration: intramuscular injection.

Name: **Sodium Stibogluconate**
Class: Pentavalent antimonial
Mechanism of action: decrease in ATP and GTP synthesis contributes to decreased macromolecular synthesis and to decreased *Leishmania* viability.
Commercial source: Albert David Ltd., India; distributed by IDA Foundation.
Product appearance: A faintly straw coloured fluid in a multi-dose brown opaque vial of 30ml containing SSG BP equivalent to 100mg pentavalent antimony in each ml (total 3g).
Administration: Given IV (slow over 5 minutes) or IM at a daily dose of 20mg/kg body weight.

7.2. Doses and treatment regimens

7.2.1 Test arms

Arm 1: Paromomycin 20 mg/kg/d IM q.d. for 14 days combined with oral miltefosine allometric dosing b.i.d. for 14 days

Arm 2: Paromomycin 20 mg/kg/d IM q.d. for 14 days combined with oral miltefosine allometric dosing b.i.d. for 28 days **(recruitment is now discontinued in this arm)**

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 <45 kg: 100 mg/day
- Patients ≥ 45 kg: 150 mg/day

PM and MF will be administered together, both starting at D1. PM dosage will be 20 mg/kg/d for 14 days administered intramuscularly, whereas MF allometric dose will be administered orally for 14 days in arm 1 (it used to be administered for 28 days in arm 2).

Patients are hospitalized for 14 days of PM and MF treatment in both arm 1 and arm 2. The MF treatment will start at the same time as the PM treatment and for arm 2 it used to continue on an out-patient basis until completion of the 28 days treatment.

Miltefosine administration

MF will be administered orally for 14 days in arm 1 and used to be administered for 28 days in arm 2.

For patients less than 30 kg, the allometric dose will be defined according to weight, height and sex as indicated in Table 1, divided in 2 administrations per day for 14 days in arm 1 or 28 days in arm 2 (not applicable now recruitment has been discontinued in arm 2).

Table 1 - Daily allometric miltefosine dose for female children based on fat-free mass

FEMALE									
HT (cm)	80-89	90-99	100-109	110-119	120-129	130-139	140-149	150-159	160
WT (kg)									
7									
8	30								
9	30			RISK OF SEVERE MALNUTRITION					
10	30	40		REFER TO THE WHO REFERENCE CURVES					
11	40	40	40						
12	40	40	40						
13	40	40	40						
14	40	40	40	50					
15	40	40	50	50					
16	40	50	50	50					
17	40	50	50	50	50				
18	50	50	50	50	60				
19	50	50	50	60	60				
20	50	50	50	60	60	60			
21	50	50	60	60	60	60			
22	50	50	60	60	60	60			
23	50	50	60	60	60	70	70		
24	50	50	60	60	60	70	70		
25	50	60	60	60	70	70	70		
26	50	60	60	60	70	70	70	70	
27	50	60	60	70	70	70	70	80	
28	50	60	60	70	70	70	80	80	
29	50	60	60	70	70	70	80	80	
30	50	60	60	70	70	80	80	80	80

Table 2 - Daily allometric miltefosine dose for male children based on fat-free mass

MALE									
HT (cm)	80-89	90-99	100-109	110-119	120-129	130-139	140-149	150-159	160
WT (kg)									
7									
8	40								
9	40				RISK OF SEVERE MALNUTRITION				
10	40	40			REFER TO THE WHO REFERENCE CURVES				
11	40	40							
12	40	50							
13	50	50	50						
14	50	50	50						
15	50	50	60	60					
16	50	50	60	60					
17	50	60	60	60					
18	50	60	60	60	70				
19	50	60	60	70	70				
20	50	60	60	70	70				
21	60	60	60	70	70	70			
22	60	60	70	70	70	80			
23	60	60	70	70	80	80			
24	60	60	70	70	80	80	80		
25	60	60	70	70	80	80	80		
26	60	70	70	80	80	80	100		
27	60	70	70	80	80	80	100		
28	60	70	70	80	80	100	100	100	
29	60	70	70	80	80	100	100	100	
30	60	70	80	80	100	100	100	100	

MF 10 mg and 50 mg strengths will be supplied through MSF Supply, Belgium, and Knight Therapeutics, US respectively in alu/alu blister packs of 56 capsules.

Administration of the daily dosage must be divided in 2 administrations, during or after a meal, to avoid direct gastrointestinal adverse effects of MF, as follows:

Total daily dose, divided:	30mg	40mg	50mg	60mg	70mg	80mg	100mg	150mg
Morning (breakfast)	2x 10mg	2x 10mg	1x 50mg	1x 50mg	1x 50mg	1x 50mg	1x 50mg	2x 50mg
Afternoon (dinner)	1x 10mg	2x 10mg	-----	1x 10mg	2x 10mg	3x 10mg	1x 50mg	1x 50mg

During the 14 days of hospitalization, administrations for all patient's arms will be directly observed by the study nurse to assure compliance. The number of capsules administered (morning and afternoon), the time of administration and any vomiting episode will be documented in a patient dispensing log. After each drug intake the patients will be observed for vomiting for 30 minutes. All vomiting episodes will be recorded. In case of vomiting within 30 minutes after intake, the dose will be re-administered. If a repeated vomiting occurs (vomiting after re-dose) the treatment will not be re-administered.

Compliance will be assessed by total dose taken by the patient. Full compliance will be considered if 90-110% of the prescribed dose is administered.

If the patient vomits a dose within 30min of drug intake and is re-dosed (without vomiting this 2nd re-dose), the re-dose will be considered for compliance. However, if the patient is not re-dosed within 30min or if s/he vomits the re-dose, this dose will not be accounted for compliance. In the case of a patient vomiting after 30min of MF intake, the dose will be accounted for compliance.

Patients in arm 2 used to continue MF intake after discharge at day 14 on an out-patient basis. Compliance during the 14 days treatment outside the hospital was assessed by collecting the empty blisters at the day 28 visit and checking for any unused drug. A diary including illustrations that is made understandable for illiterate subjects was provided to these patients in order to guide them in their treatment schedule at home. Any episode of vomiting reported by the patient was captured by the clinician at day 28 visit. In case of multiple episodes of vomiting, patients were instructed to come to the hospital for an assessment in an unscheduled visit. Compliance will be further assessed through cross-check with PK data.

Paromomycin administration

PM will be given for 14 days in both arm 1 and arm 2. Exact daily dose of 20 mg/kg will be calculated using patient's body weight and will be administered once-a-day with intramuscular injection into the buttock muscle.

PM sulfate will be supplied through IDA Foundation, Holland, in boxes of 10 amber-colored glass ampoules each, containing an equivalent 750 mg of PM base in 2 ml (375 mg/ml).

All administrations will be directly observed by the study nurse or clinician to assure compliance.

Treatment interruption

MF treatment in arm 1 (14 days) will last at least 13 days in order to be considered 90% compliant. In case of treatment interruption longer than 24 hours, missing doses will be compensated after day 14.

MF treatment in arm 2 (28 days) could be temporarily interrupted (due to AEs or at discretion of investigator) and resumed if this interval was no longer than 72hs. In this case, the total administered dose over a total of 25 days was still considered within 90% range of full compliance, therefore it was not considered a compliance deviation. The missing doses was not compensated after the final dose on day 28, and the

schedule of assessments would remain the same.

If treatment needs to be interrupted for > 72hs due to an AE related to MF, or if treatment needs to be interrupted due to an AE related to PM or SSG, rescue treatment will be considered, at the discretion of the investigator.

7.2.2 Reference arm

Arm 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

SSG will be given IM/IV at a dose of 20 mg/kg once daily for 17 days. PM base will be administered IM at a dose of 15 mg/kg once daily for 17 days. The dosing regimen will be the combination of both SSG and PM as described above.

7.3. Study treatments labelling, packaging

Commercially available MF, PM and SSG will be used. Trial specific labels will be applied on the boxes prior to use with the following statement *"For Clinical Trial use Only"* and will include protocol code, Sponsor and PI contact details as well as direction for use.

7.4. Accountability

Study medications must be kept in a locked room that can be accessed only by the pharmacist or designated personnel by the investigator. The study medications must not be used for other purposes other than this protocol. Under no circumstances the investigator or site staff may supply study medications to other investigators or sites, or allow the medications to be used other than as directed by this protocol without prior authorization from DNDi. Under no circumstances can the study specific products (appropriately labelled) be replaced by other commercially available product (not specifically trial labelled).

Specific study forms for drug accountability will be designed to allow for adequate records on receipt, use, return, loss, or other disposition of medications. These accountability forms must be maintained by the study pharmacist or designated personnel and stored in the locked room with the study medication.

7.5. Storage

Study drugs must be kept in a locked cabinet and/or in a room with restricted access, under the control of the study pharmacist.

Temperature of the storage location will be monitored daily and recorded in a temperature log. In the event of temperature excursions below or above the allowable range, site staff will inform the study monitor.

Miltefosine: MF must be stored at 20-25°C; excursions permitted between 15-30°C. The product should be stored in the original package and protected from moisture. The shelf life is 5 years. Packaging should be undamaged prior to use.

Paromomycin: The product should be stored below 30°C and protected from light. Partially used ampoules must not be stored for future patient use. The shelf life is approximately 3 years. Packaging should be undamaged prior to use.

Sodium Stibogluconate: The product should be stored below 30°C and protected from light. The shelf life is 3 years. The contents should not be used for more than one month after opening.

7.6. Blinding and procedures for unblinding

This is an un-blinded open label study.

7.7. Concomitant treatments

All medications required for concomitant conditions should be postponed until after the end of treatment for VL, unless warranted by immediate medical need. Common conditions such as malaria and respiratory tract infections should be treated prior to starting VL treatment.

Details of all concomitant medications used during the study will be recorded in the patients' charts and in the CRF, with reason for use and dates of administration. Concomitant medications will be coded using the WHO Drug Dictionary Enhanced.

Drugs that prolong the QT interval (e.g. quinine) should be avoided while SSG is being administered. If it is urgently required to give such drugs, SSG should be stopped; otherwise alternatives should be sought (e.g. artemisinin derivatives). For MF, there are no known interactions with other commonly used medications, though this cannot be excluded. While giving PM, the use of other aminoglycosides must be avoided (e.g. gentamicin).

7.8. Rescue treatment

In case of treatment failure or intolerability (treatment must be permanently interrupted), rescue treatment will be provided to the patient, at the discretion of the study physician and based on the national VL treatment guidelines. For patients in arm 1 and 2, rescue treatment is expected to be SSG/PM (SSG 20 mg/kg/day, PM 15 mg/kg/day) or AmBisome multiple dose (30 mg/kg total dose). In arm 3, rescue treatment is expected to be AmBisome multiple dose (30 mg/kg total dose).

Patients will be given rescue treatment if they have:

- failed to respond to MF/PM treatment or to SSG/PM reference treatment within the treatment period (lack of improvement or worsening of the clinical signs and symptoms of VL).
- failed to respond to MF/PM treatment or to SSG/PM reference treatment at the EOT assessments (D28). This will be reflected by positive parasitological assessment and/or lack of improvement or worsening of the clinical signs and symptoms of VL.
- failed to tolerate trial medication / occurrence of adverse event(s) during receipt of trial drugs that requires permanent treatment interruption, and rescue treatment is indicated by the investigator (see section 9).
- relapsed, *i.e.* recurrence of symptoms, signs and presence of parasites after EOT

(D28) during the follow up period
- developed severe post kala azar dermal leishmaniasis (PKDL) that necessitates rescue treatment (see section 8.3.4).

8. Study Assessments

8.1. Timing of Assessments

Assessments will be carried out at Screening period (day -7 to 0) and on days 1, 3, 7, 14, 21, 28, 56 and 210. It will include clinical, parasitological, haematological, biochemistry, safety, pharmacokinetic and pharmacodynamics assessments.

A table with the schedule of events is listed under section 5.

All patients will be hospitalized for screening/baseline procedures until completion of day 14 (arm 1 and 2) and day 17 (arm 3) procedures (approximately 21-24 days). Day 28, 56 and 210 assessments will be conducted as outpatient visits.

Assessments performed during study visit at D28 will have allowable window of +1day, due to critical PK assessments at this EOT visit. Therefore, it is acceptable to have patients readmitted at the hospital the day before (D27). In the case of patients coming from very remotes areas, mobilization will be strengthened and it will be allowed to readmit those patients 2 to 3 days before D28 assessments.

All other assessments performed during treatment period (D1 until < D28) will have an allowable window of +/-1 day. In the follow-up period, visit windows will be: day 56 (±7 days) and day 210 (±14 days).

8.2. Baseline Assessments

The following baseline assessments will be done during screening (and after obtaining patient consent) in order to confirm VL diagnosis and verify inclusion and exclusion criteria.

- Demographic data including age and sex
- Medical history including relevant past history, previous VL episode, concomitant medications and medication taken in previous 14 days
- Clinical examination: Physical examination including clinical signs and symptoms of VL, PKDL assessment, body weight, height, vital signs (Temperature, Heart rate and Blood pressure), spleen and liver size
- Nutritional status: severe malnutrition will be defined as follows*:
- for children aged <5 years with weight-for-height WHO reference curves by sex, z score <-3;
- for patients 5-18 years with BMI-for-age WHO reference curves for sex, z score < -3;
- for adults ≥19 years as BMI < 16
- ECG
- Audiometric test
- Clinical safety laboratory evaluations:

- Haematology: Haemoglobin, total WBC with differential, platelets
- Biochemistry: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), total bilirubin (TBIL), Creatinine (CRE), Albumin (ALB)
- Urine pregnancy test
- HIV test – routine counselling for HIV test will be given for patients' parents, as part of the study informed consent procedure interview or a separate consent will be requested, according to the local requirements. Testing will be done according to national guidelines and undergo a single rapid diagnostic test for the purposes of the clinical trial. If the RDT for HIV is positive, the patient must be excluded from participating in the clinical trial and receive current standard treatment for VL and further assessment and treatment for HIV according to the national HIV treatment Guidelines.
- Spleen, bone marrow or lymph node aspirate for parasitology (microscopy and culture, see additional information in section *8.3.2. Parasitological assessment*) and pharmacodynamics assessment (qPCR)**
- Blood sample for pharmacokinetics and pharmacodynamics assessment

** Note: for Ethiopia, severe malnutrition will be defined as follows:*

- *For patients 4-18 years, MUAC cut-off based on the following MUAC-for-height reference table:*

Height	***MUAC cut-off for severe malnutrition***
65-<110 cm height	*< 115 mm*
110- <120 cm	*< 120 mm*
120-<130 cm	*< 125 mm*
130-<140 cm	*< 135 mm*
140-<150 cm	*< 145 mm*
150-<160 cm	*< 150 mm*
160-<170 cm	*< 155 mm*
≥170 cm	*< 160 mm*

- *For patients >18 years, MUAC < 170 mm*

MUAC is the circumference of the left upper arm and is measured at the mid-point between the tips of the shoulder and elbow.

*** Note: microscopy is mandatory. Culture and qPCR material to be collected if there is remaining material after preparing the slides.*

8.3. Assessment of Efficacy

8.3.1 Clinical assessment of VL

The clinical evaluation* will involve measuring temperature (axillary), spleen size, liver size, body weight at screening, on day 1, 3, 7, 14, 21**, 28 and at all follow-up assessments on day 56 and 210. In addition, the following VL symptoms will be

systematically checked at each study visit: fever, abdominal swelling, loss of appetite, diarrhea, coughing, epistaxis or other bleeding signs, jaundice.

The size of the liver will be measured in the midclavicular line for its total span; the spleen size will be measured from the left costal margin on the anterior axillary line to the tip of the spleen medially.

** Note: for Ethiopia, clinical evaluation will include measurement of MUAC at screening and on day 28, 56 and 210.*

*** Note: D21 visit was only applicable to the subset of patients allocated to PK sampling in arm 2 (now discontinued).*

8.3.2. Parasitological assessment

Parasitological assessment will be done at baseline and at day 28 through spleen or bone marrow aspirate and microscopic examination.

Spleen aspirate should not be performed if platelets count is < 40,000/mm^3, Hb ≤ 5g/dL, if the patient has signs of bleeding, jaundice, or if the spleen is not palpable. In these cases, bone marrow aspiration is recommended.

In addition, sites performing splenic aspiration for parasitology must also perform prothrombin time as an additional compulsory screening test to determine whether it is safe to proceed with splenic aspiration. If there is more than a 5 second difference between the normal control value and prothrombin time result obtained, the patient is not eligible for splenic aspiration at that point in time. In this case, the investigator should, either,

a. Administer Vitamin K for 3 days and repeat the prothrombin time test on day 4. If prothrombin time result is within acceptable range (not more than 5 seconds difference from normal control), and all other parameters for splenic aspiration are within the required range, then a splenic aspirate can be done

OR

b. Perform bone marrow aspiration instead

If the site does not have the capability to perform the prothrombin time test, splenic aspiration should not be performed, and bone marrow aspiration should be performed instead.

Due to the risk of hemorrhage post spleen aspiration, the patient must remain under observation. Record pulse and blood pressure every half hour for 4 h, then every hour for 6 h. Patient must remain in bed for 12 hours.

Patient monitoring post-splenic aspiration should strictly be observed as per the study protocol and in-country national VL treatment guidelines. Any suspicion of bleeding such as a drop in blood pressure, rising heart rate, abdominal discomfort or pain, or any other sign as per investigator's judgement should be followed by immediate emergency response measures as per national VL treatment guidelines.

During the follow-up visits at day 56, day 210 or unscheduled visit, parasitology will be performed when clinically indicated, *i.e*. anytime if suspect of relapse (reappearance of symptoms and signs of VL).

Aspirates will be smeared on slides, stained and read under microscope.

For spleen aspirate, slide reading will be graded as positive or negative according to the standard logarithmic criteria as defined in Table 3.

Table 3 - Standard logarithmic criteria for Visceral Leishmaniasis parasitological diagnosis

Count Oil Immersion x 100	
6+	> 100 parasites per field
5+	10-100 parasites per field
4+	1-10 parasites per field
3+	1-10 parasites per 10 fields
2+	1-10 parasites per 100 fields
1+	1-10 parasites per 1000 fields
0	0 parasite per 1000 fields

Optionally, if there is sufficient tissue aspirate sample remaining after the slide has been prepared, this will be processed and used for pharmacodynamics analysis (see section 8.4.2.) and culture.

8.3.3. Clinical Lab assessments related to VL evolution

Hematology parameters are commonly affected in a patient with VL. At baseline, the patient is likely to present anemia, low WBC and possibly low platelets.

Exclusion criteria are defined as not to include in the clinical trial patients with severe anemia (Hb< 5g/dL). In addition, Hb level, platelets and WBC counts will be assessed by the investigator together with the clinical presentation and other laboratory parameters to judge if patient has signs of severe VL disease, requiring AmBisome therapy.

Hematology parameters must be recorded at screening, D3, D7, D14, D28, D56 and D210. It is expected that the hematological parameters recover with time after treatment.

Albumin will also be assessed before and after treatment, at screening, Day 28 and Day 210.

8.3.4. Post-kala-azar dermal leishmaniasis (PKDL)

Patients will be monitored closely for post-kala-azar dermal leishmaniasis (PKDL) through the course of the study. Diagnosis will be made clinically based on the typical appearance and distribution of the rash. Presence of PKDL will be assessed at screening, on day 28 and during follow-up assessments on day 56 and day 210.

Grading will be noted during the assessment times as follows:

- Mild (Grade 1): lesions mainly on the face and head, with others scattered on arms, chest and back
- Moderate (Grade 2): the upper part of the chest and arms are also affected
- Severe (Grade 3): lesions may be found on the whole body.

Patients who develop severe PKDL (grade 3) or have mucosal and/or eye involvement (any grade) will require treatment with rescue medication at the discretion of the attending physician.

8.4. Pharmacokinetics and Pharmacodynamics Assessments

8.4.1. Pharmacokinetic Assessments

Patients in arm 1 will have pharmacokinetics measurement of MF at D14, whereas all patients in arm 1 and 2 will have PK 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: originally 40 in each MF/PM combination arms, with selection of 20 pediatric (≤12 yrs) patients and 20 adolescent/adult patients (>12 to ≤50 yrs). Because arm 2 is discontinued, the number of patients enrolled in this arm in the PK/PD intensive cohort will most probably not reach 40, however samples collected will still be analysed. Enrollment in this intensive cohort will be limited to specific sites located in Sudan and Kenya and as far as possible should be balanced between the two countries. MSF sites will not participate to the enrollment in the intensive PK/PD cohort. 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. Due to the PK nonlinearities observed in pediatric patients receiving the allometric MF dosing (LEAP0714), the inclusion of children in this PK sub-study is important and therefore the aim is to have equal numbers of patients below 12 years and above 12 years included in the PK sub-study, to allow for comparison. For PM, we have included a sparse sampling scheme to enable assessment of pharmacokinetic profiles, with subsequent use of population PK modelling to analyze the data. PM PK will be assessed on both day 1 and day 14 of treatment to assess the influence of changing patient characteristics during the treatment period (e.g. increasing body weight, reduced liver damage, etc.) on the PK of PM and to assess potential accumulation of the drug during the treatment period.

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 intensive cohort.

To reduce the number of total samples/blood volume per patient in the intensive cohort, a mixed sampling approach will be performed. Patients will be randomly allocated to sparse sampling schemes to allow for a pooled PK analysis.

As much as possible, a single blood draw should be performed per study visit to avoid multiple vein punctures. Sample for both MF and PM PK will be in general a 2mL EDTA whole blood sample/time point. For time-points where only MF PK is to be assessed, also 2 ml EDTA whole blood sample/time point will be collected to accommodate with the lowest volumes of tubes currently available on the market.

The pharmacokinetics of both PM and MF will be determined using validated liquid chromatography–tandem mass spectrometry (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.

Samples will be stored on site at minimally -20°C and regularly shipped under frozen conditions to the respective capitals for shipment to Amsterdam, the Netherlands. Samples will be analysed for their MF/PM content at the Bioanalytical Laboratory at the Netherlands Cancer Institute – Antoni van Leeuwenhoek Hospital, following a validated procedure using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The development and validation of the assay for MF has previously been published (Dorlo et al., 2008). A bioanalytical assay for paromomycin in human plasma has been described in literature previously and will be validated during the progressing review period and implementation of this protocol.

8.4.1.1. Paromomycin pharmacokinetic assessments

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. The screening sample can be collected at any day during screening period. PM PK sample at D1 and D14 will be collected at 0, 1, 2, 4, 8, 24 hrs post-dose.

In order to minimize blood volume collected, each patient will be randomly 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.

Time of drug administration and time of PK samples collection have to be recorded systematically.

8.4.1.2. Miltefosine pharmacokinetic assessments

MF PK assessments will be performed at D14 for subjects in arm 1 and at D28 and D56 for all subjects in arm 1 and 2 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

used to be done at D21 in arm 2 only (not applicable anymore in this protocol version). The screening sample can be collected at any day during screening period or before the administration of the 1st dose at D1.

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). The 8h MF PK sample will be collected always before the 2nd daily dose. D7 and D21 (arm 2 only, not applicable in this protocol version) PK samples will be collected prior to the morning dose. D28 PK sample used to be collected prior to the morning dose in patients allocated to arm 2 and will be collected in the morning in patients allocated to arm 1. In the follow-up period (D56), PK sample shall be collected in the morning. Time of drug administration and time of PK samples collection have to be recorded systematically.

8.4.2. Pharmacodynamic Assessments

Parasite load in blood and tissue aspirate (bone marrow or spleen): PD assessment will be based on real time quantitative polymerase chain reaction (qPCR) for *L. donovani* in EDTA blood or tissue aspirate, based on the amplification of kinetoplastid DNA (kDNA) (other *Leishmania* DNA targets may also be considered), to assess the parasite clearance over time during the treatment with two combination regimen of PM and MF or treatment with SSG/PM.

All patients will have qPCR in tissue and blood at screening and at Day 28 assessment; and during follow-up if there is suspicious of relapse.

In addition, the patients (40 in arm 1 and probably fewer patients in arm 2 now this arm is discontinued) in the intensive cohort who consent to have multiple sampling for PK will also have qPCR blood samples collected at D0, 3, 7, 14, 28, 56 and 210.

Approximately 1 ml EDTA whole blood will be immediately separated from blood EDTA samples taken (e.g. for Complete Blood Count or PK analysis) for the qPCR analysis. The blood samples and also the remainder of bone marrow/spleen aspirate sample (optional) will be immediately diluted with DNA stabilizing buffer solution (L6 buffer) and can then be stored up to 2 years at frozen temperature.

Furthermore, additional relevant PD markers will be considered for analysis in order to monitor treatment effect.

8.5. Assessment of Safety

Safety of the different treatment regimens will be assessed through routine monitoring of adverse events. At each study visit, the patients will be enquired about current adverse events or any events observed during the period previous to the visit. In addition, evaluation of hematology and blood chemistry parameters, regular measurement of vital signs and physical examinations will be made at scheduled follow-up visit.

Audiometric test will be performed at baseline and on days 14, 28 and 210 for all patients.

ECG will be performed at baseline and on day 14 in all arms, and in addition on days

7 and 28 in the reference arm SSG/PM. SOPs will be developed to guide investigators on audiometry and ECG assessments.

Urine pregnancy tests will be performed in woman of child-bearing potential at baseline to exclude any pregnancy, and on D28, D56 and D210 visits to monitor eventual unexpected pregnancy.

HIV test will be repeated at the D210 visit only for patients in Gondar and Abdurafi sites, due to high prevalence of HIV/VL co-infected patients (15%) in this specific population.

The frequency and severity of AEs will be described, as well as frequency of SAEs or AEs that lead to treatment discontinuation. See section 8.6 for AE definition and reporting.

8.5.1. Laboratory examinations

Hematology parameters hemoglobin, WBC with differential and platelets will be analyzed at screening, on days 3, 7, 14, 28, 56 and 210.

Biochemistry parameters ALT, AST, TBIL and CRE will be analyzed at screening, on days 3, 7, 14 and 28.

Samples will be analyzed at the local laboratory using standardized equipment.

Approximately 3 ml of blood will be collected at each visit: 1 ml in EDTA for the hematology and 2 ml in dried tube for the biochemistry.

Lab parameters abnormal values will be assessed for clinical significance and will be graded according to CTCAE v4.03 (see section 8.6.7).

8.6. Adverse event definitions and reporting

8.6.1. Adverse Event definition

Any untoward medical occurrence in a clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

It can therefore be any unfavourable and unintended sign (e.g. an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Definition of an AE includes worsening (in severity and frequency) of pre-existing conditions (“Medical history”) before first Investigational Medicinal Product (IMP) administration and abnormalities of procedures (i.e. ECG, X-ray…) or laboratory results which are assessed as “clinically significant” (see details in section 8.6.2).

What is not an AE?

- Medical conditions present at the initial study visit that do not worsen in severity or frequency during the study are NOT considered as AE.
- Symptoms, exacerbation or worsening of the studied disease will NOT to be considered as AE nor captured on the AE page of the CRF if consistent with the anticipated natural progression of the disease (overall and for this given subject).
- Lack of efficacy of the IMP is NOT considered as AE.

8.6.2. Assessment of laboratory abnormalities

For every laboratory assessment, the investigator will evaluate if the lab test is normal or abnormal. If abnormal, the investigator will assess if this finding is clinically significant or not. If a lab parameter is abnormal and clinically significant, it should be reported as an adverse event (AE).

An abnormal lab test must be compared with the previous value.

An AE is a new event after the administration of the 1st dose of the study drug or a worsening in the condition (in the case of lab tests, increase in severity by CTCAE v4.03) which is judged clinically significant by the investigator.

Laboratory abnormalities should be assessed as “clinically significant” (and therefore have to be reported as an AE) only if they meet AT LEAST ONE of the following conditions:

- The abnormality suggests a disease and/or organ toxicity **AND** this abnormality was not present at the screening visit or is assessed as having evolved since the screening visit

- The abnormality results in discontinuation of the study drug

- The abnormality requires medical intervention or concomitant therapy

Lab parameters which are classified with regards of severity as CTCAE v4.03 Grade 3 or Grade 4 must be carefully assessed, as they are likely to require medical intervention and/or be life-threatening in terms of severity.

For the purpose of this protocol, **all clinically significant Grade 3 and Grade 4 lab abnormalities must be reported as an AE.**

When reporting an abnormal lab, a clinical diagnosis should be recorded rather than the abnormal value itself, if available (for example, “hypokalemia” rather than “decreased potassium levels”). Furthermore, lab abnormalities which present with clinical signs and symptoms will also be considered clinically significant. In these cases, again the adverse event should be recorded as the syndromic clinical diagnosis rather than clinical signs and symptoms (*ex.* acute pancreatitis instead of each finding separately: high levels of amylase, high levels of lipase, abdominal pain and vomiting).

For any abnormal clinically significant lab value, the investigator will carefully assess if the event is due to the natural disease progression (e.g. anaemia during the first days of treatment). If the abnormality is associated with the anticipated VL disease symptoms, it does not need to be reported as an AE, unless if it meets the criteria of a

clinically significant event.

8.6.3. Serious Adverse Event

An adverse event will be defined as serious if it

- **results in death,**

  i.e. causes or contributes to the death.

- **is life-threatening,**

  in this context refers to an AE/Adverse Reaction (AR) in which the patient was at risk of death at the time of the AE/AR; it does not refer to an AE/AR that hypothetically might have caused death if more severe.

- **requires in-patient hospitalisation or prolongation of existing hospitalisation**,

  i.e. the AE requires at least an overnight admission or prolongs a hospitalization beyond the expected length of stay.

  In this protocol, the patient is expected to be hospitalized for 14 days, with discharge expected on D15. If the patient presents an AE that requires hospitalization for more than 24hs after D15, this event will be considered an SAE. However, if the patient remains hospitalized for social reasons, it will NOT be considered as an SAE.

  Hospital admissions or prolongation of hospitalisation for surgery planned before study entry, for social reasons, for any elective surgery (i.e. plastic surgery) or for normal disease management (including treatment adjustment) are NOT to be considered as SAE according to this criterion (i.e. if the protocol or the standard management of the disease under study requires planned hospitalization).

- **results in persistent or significant disability or incapacity**,

  i.e. the AE resulted in a substantial disruption of the subject's ability to conduct normal activities.

- **is a congenital anomaly / birth defect,**

  i.e. an adverse event outcome in a child or foetus of a subject exposed to the Investigational Medicinal Product (or marketed medicinal product) before conception or during pregnancy.

- **is an important medical event, i.e. is medically significant.**

  Medical and scientific judgment should be exercised in deciding whether other situations should be considered serious events/reactions, such as important medical events that might not be immediately life-threatening or result in death or hospitalization but might jeopardize the patient or might require intervention to prevent one of the other outcomes listed above.

In addition, any suspected transmission via a medicinal product of an infectious agent is also considered a serious adverse event/reaction.

Note: **SAE onset date** is the SAE start date or date a pre-existing AE becomes serious.

8.6.4. Eliciting Adverse Event information

The investigator is required to report all directly observed adverse events and all adverse events spontaneously reported by the trial subject using concise medical terminology. In addition, each trial subject will be questioned about the occurrence of adverse events at each study visit (scheduled and non-scheduled) since the time of the last study visit, as well as follow-up from previously recorded adverse event.

8.6.5. Management of Adverse Events

All adverse events will be managed by the investigators according to local treatment guidelines. The sites are equipped with appropriate resources to treat adequately the adverse events. However, in case the patient cannot be managed on site, he/she will be referred for specialized care in the nearest referral hospital.

8.6.6. Adverse Event reporting period

The adverse events reporting period begins upon subject enrolment in the trial (after signature of informed consent) and ends at the end of the subject participation in the trial (D210 follow-up visit).

In this protocol, the reporting period is different for AEs and for SAEs:

- Non-serious adverse events: All non-serious AEs will be reported upon administration of the first dose of trial medication until the end of subject participation in the trial (including post-treatment follow-up period up to D210). Furthermore, non-serious AEs that occur in the *screening period* (from signature of the informed consent) AND are judged as study-related (e.g. hematoma due to venipuncture) will be reported in the AE CRF.

- Serious adverse events: All SAEs must be reported upon subject enrolment in the trial (after signature of informed consent during screening period) until the end of the subject participation in the trial (including post-treatment follow-up period up to D210).

- Screening failure: beyond the date of screening failure (to be recorded), only serious study-related events will be followed-up.

  All adverse events that occur during the adverse event reporting period specified in the protocol must be reported to DNDi, whether or not the event is considered medication related. In addition, any adverse event that occurs subsequent to the adverse event reporting period that the investigator assesses as possibly related to the investigational medication should also be reported as an adverse event.

8.6.7. Adverse Event reporting requirements

Information on adverse events must be evaluated by a physician. Each adverse event is to be classified by the investigator as serious or non-serious. This classification will determine the reporting procedure for the event to DNDi and Health Authorities/Ethics Committees (as per local regulatory requirements).

All serious adverse events (SAE) are to be reported immediately (within 24 hours of awareness of SAE by the investigator) to DNDi (SAEMFPMstudy@dndi.org), using the SAE report form. This includes a description of the event, onset date and seriousness criteria, duration, severity, relationship to study drugs, outcome, measures taken and all other relevant clinical and laboratory data. The initial report is to be followed by submission of additional information (follow-up SAE form) as it becomes available. Any follow-up reports should be submitted as soon as possible, and if possible within 5 working days.

In addition to immediately reporting SAEs to DNDi, Investigators are responsible for reporting SAEs occurring at their site to the Ethics Committee (IEC), and any periodic safety reporting if applicable, in compliance with the local regulatory requirements.

Serious adverse events should also be reported on the clinical trial adverse event case report form (CRF). It should be noted that the form for reporting of SAE (SAE form) is not the same as the adverse event section of the CRF. Where the same data are collected, the two forms must be completed in a consistent manner, and the same medical terminology should be used.

Non-serious adverse events are to be reported on the CRF, including description of the event, onset date, duration, severity, seriousness, relationship to all study drugs, actions taken and outcome. In the CRF, a given adverse event will be recorded only one time per patient, and the severity recorded will be the maximum level reached. If several distinct episodes of the same condition occur, their number will be recorded in the CRF.

8.6.8. Grading of Adverse Event severity

Severity is a clinical determination of the intensity of an AE. The severity for an AE should be graded using the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE, version 4.03).

In case of AEs that are not described in the CTCAE v4.03, the investigator will use the terminology MILD, MODERATE, SEVERE, LIFE-THREATENING or DEATH to describe the maximum severity of the adverse event as follows:

MILD (grade 1) The subject is aware of the event or symptom, but the event or symptom is easily tolerated (e.g. no reduction in daily activities is required)

MODERATE (grade 2) The subject experiences sufficient discomfort to interfere with or reduces his or her usual level of activity.

SEVERE (grade 3) Significant impairment of functioning: the subject is unable to carry out usual activities and/or the subject's life is at risk from the event

LIFE-THREATENING (grade 4) The subject is at risk of death at the time of the adverse event. It does not refer to an AE that hypothetically might have caused death if more severe (Life-threatening consequences, urgent intervention required)

DEATH (grade 5) Death related to AE

This information on AE grading will be entered in the adverse event CRF.

It is to be noted the distinction between severity (intensity) and seriousness (regulatory definition) of adverse events. A severe adverse event is not necessarily a serious adverse event.

8.6.9. Adverse Event causality assessment

For both serious and non-serious adverse events, the investigator is required to assess if there is a causal relationship between the adverse event and all the study drugs, i.e. to determine whether **there is at least a reasonable possibility** that one of both study drugs caused or contributed to the adverse event. This means that there are facts and arguments to suggest a causal relationship.

To help investigators with the decision binary tree in the evaluation of causality, the CIOMS VI group recommends that investigators be asked to consider the following before reaching a decision:

- Medical history
- Lack of efficacy/worsening of existing condition
- Study medications
- Other medications (concomitant or previous)
- Withdrawal of study medication, especially following trial discontinuation / end of study medication
- Erroneous treatment with study medication (or concomitant)
- Protocol related procedure

Using this information the investigator will classify each adverse event as:

Not related: **There is** no reasonable possibility **of causal relationship between an AE and study drug**

Related: There is **at least a reasonable possibility** of a causal relationship between an AE and a study drug. This means that **there are facts (evidence) or arguments** to suggest a causal relationship.

The decision to suspend, and resume treatment or to permanently interrupt treatment due to an adverse event will be left to the clinician in charge.

8.6.10. Exposure in utero

All female patients of child bearing age will be excluded unless they agree to undergo a pregnancy test and explicitly consent to take an acceptable method of contraception (i.e. Depo-Provera). Urine pregnancy tests will be performed in woman of child-bearing potential at baseline to exclude any pregnancy, and on D28, D56 and D210 visits to monitor eventual unexpected pregnancy. All pregnant women will be excluded from the study. This criterion was set because of the teratogenic potential of MF (demonstrated in rats). The effective contraception should be used for a minimum period of 5 months after end of treatment with MF. Therefore, Depo-Provera injection will be required before treatment onset and around day 90 to cover for the entire exposure period.

However, in the unlikely event that any trial subject becomes or is found to be pregnant while receiving any of the study treatment drugs or during follow-up period, the investigator must submit the event on a Pregnancy report form. The information submitted should include the anticipated date of delivery.

The investigator will follow the subject until completion of the pregnancy or until pregnancy termination (i.e., induced / spontaneous abortion). The investigator will provide pregnancy outcome information on a Pregnancy outcome form.

A pregnancy is not an SAE.

In the case of a live birth, a medically qualified person should assess the infant at the time of birth and submit a Child report. An SAE should be declared in the case of unfavorable pregnancy outcome (abortion, still birth) or congenital abnormality (in addition to the Child report).

8.6.11. Adverse event follow up

All adverse events should be followed until they are resolved or the investigator assesses them as chronic or stable or the subject participation in the trial ends (i.e., until a final report is completed for that subject).

In addition, all serious adverse events and those non-serious events assessed by the investigator as possibly related to the investigational drug must continue to be followed even after the subject participation in the trial is over. Such events should be followed until they resolve or until the investigator assesses them as “chronic” or “stable.” Resolution of such events is to be documented on the CRF and SAE form.

9. Withdrawal criteria

A subject should be withdrawn from the study treatment if, in the opinion of the investigator, it is medically necessary, or if it is the wish of the subject.

If a subject does not return for a scheduled visit, every effort should be made to contact the subject.

In any circumstance, every effort should be made to document subject outcome, if possible.

If the subject withdraws consent, no further evaluations should be performed and no attempts should be made to collect additional data, with the exception of safety data,

which should be collected if possible and in accordance with patient consent.

If a subject withdraws from the study, the reason must be noted on the CRF.

If a subject is withdrawn from the study because of a treatment limiting adverse event, thorough efforts should be made to clearly document the outcome of AE.

9.1. Rules in case of treatment suspension or interruption

In the event that treatment is interrupted (e.g. due to an adverse event), the decision to resume treatment will be taken by the site Principal Investigator.

If treatment interruption lasts > 72hs due to an AE related to MF, or if treatment needs to be interrupted due to an AE related to PM or SSG, rescue treatment will be considered, at the discretion of the investigator.

Once rescue treatment is given, the patient will be considered a treatment failure, and will be withdrawn from the study.

If rescue treatment is not indicated, the patient may continue to participate in the study, and perform the scheduled visits and procedures.

9.2. Rules for permanently interrupting study treatment

If a subject is withdrawn from the study before the full course of the treatment is completed, the physician must make all necessary arrangements to ensure that the subject receives the appropriate treatment for the relevant medical condition (e.g. with drug/s currently recommended by the national policy).

9.3. Subject withdrawal from the study and subject replacement

Subjects withdrawn from the study will not be replaced.

10. Data Analysis and Statistical Methods

10.1. Sample size determination

Samples size calculation is based on the efficacy (definitive cure) at 6 months (D210) which is the primary endpoint and main assessment criterion of interest.

The initial sample size assumptions were based on 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) was 173 patients per arm rounded to 175. With the provision for lost to follow-up (LTFU), the original sample size for each arm was 192 (total of 576 patients).

In this revised protocol, the analysis will remain as planned, but comparison will be performed only between one investigational arm (arm 1 MF/PM 14 days) and the standard of care SSG-PM. Because the number of comparisons is now limited to one, there is no multiplicity of testing issue and therefore, no need for adjustment of type

one error if the change in the protocol is not linked to an interim analysis or a shift to an adaptive design such as “drop the looser”. Reasons for stopping a dose regimen is not based on significant data but rather related to the paucity of patients fulfilling inclusion criteria. Moreover, the number of patients who reached the primary time point was around 10% of the planned sample size and considering the expected success rate (93%), the number of observed failures cannot be informative unless it would have been a major discrepancy with expectation. Even if collected data are considered informative, the use of O’Brien Fleming curve in the alpha spending function when 10% of the sample size was available indicates that the fraction of alpha spent at the potential interim analysis becomes quite negligible at the final analysis. Consequently, no adjustment of alpha was considered necessary in the current situation. Using same assumptions for efficacy and non-inferiority margin, a power of at least 80%, and considering an alpha of 0.025 (one-sided, no adjustment for multiplicity) the required sample size is 153 patients per arm. A 10% provision for potential LTFU to ensure a power of at least 80% brings the sample size to 170 patients per arm (1 and 3).

The total number of patients included in the trial will be approximately 420, taking into account the number of patients randomized to treatment arm 2 before discontinuation of this arm (approximately 80 patients).

10.2. Definition of study populations included in the analysis

The following patient populations will be defined for analysis:

- Intention-To-Treat (ITT): all randomized patients in the arms to which they were randomly assigned regardless of their adherence with the inclusion-exclusion criteria, the treatment they received, and subsequent withdrawal from treatment or deviation from the protocol.

- 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 (PP) 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.

The primary population for efficacy analysis at D210 will be the modified intention to treat population (mITT or FAS). Furthermore, per-protocol set of patients and the set of completers sensitivity analyses will be performed.

All patients enrolled in the study and who have been administered the first dose of study medication will be included in the safety analysis.

For the secondary endpoint on PK, all subjects exposed to at least one dose of the medication and who have a PK sample collected will be included in the analysis.

Patients' data randomized in arm 2 (PM 14 days/MF 28 days), which recruitment is now discontinued, will be summarized in a descriptive analysis, but not included in the comparative efficacy analysis.

10.3. Subject Disposition

The number of patients who were screened, failed screening, randomized, completed the treatment period, completed the follow-up, early terminations and reasons for early termination of study will be summarized by number of patients (*n*) and percentages (%).

10.4. Baseline

Routine baseline characteristics including age, sex, height, weight, BMI for age*, and symptoms (fever, weight loss, epistaxis, etc.) will be documented and summarised as appropriate according to data variable type (e.g. mean for normally distributed continuous data).

** Note: for Ethiopia, MUAC will be documented in addition to BMI for age*

10.5. Treatment Compliance

All test drugs will be supervised on administration by study medical staff. The dose given will be recorded in the patient dispensing log and in the CRF. Full compliance will be considered if 90-110% of the prescribed dose is administered.

10.6. Efficacy Analysis

Primary endpoint:

- Definitive cure or treatment success:

- absence of signs and symptoms of VL at D210,
- no rescue treatment 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;
- 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), did not relapse before D210 (no rescue treatment, 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 treatment.

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 comparison: MF-PM 14 days vs SSG-PM.

The significance limit will be 0.025 (one-sided). 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 $1 - 2P_R$. Because the Blackwelder test is based on binary data, the value (1 – 2Pr) will be rounded to the lower whole number (number of successes in patients with unconfirmed status) and added to the number of successes in other patients.

The relapse rate (P_R) is multiplied by two to impose a penalty when the status at D210 is unknown. Considering that study arms 1 and 3 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 MF/PM 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: $P_S = 1 - P_R$).

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.

<u>Justification of the non-inferiority margin</u>

The non-inferiority margin of 7% has been defined after consultation with experts. It is justified by the following advantages of the tested combination arm over the reference arm: replacing SSG with oral MF will improve treatment safety by removing the toxic antimonial component of the current SSG-PM treatment; it will be a more patient-friendly treatment by replacing one of the two daily injections with an oral administration; and hospitalization time will be reduced by at least 3 days. Finally, the new combination will ensure suitability in remote areas where VL occurs.

<u>Shift from non-inferiority to superiority</u>

If the tested combination is significantly non-inferior to the reference arm, the test of superiority will be performed (Likelihood ratio test) without adjustment of type error.

The result of this test as well as the 95% confidence interval of the excess success rate will be used to assess the benefit risk of the tested combination with regards to the reference arm but not to claim the superiority. The analysis will be the same as the primary analysis after replacing the non-inferiority test by the superiority.

Interim analysis

No interim analysis (IA) will be performed in this trial.

10.7. Safety Analysis

All patients enrolled and who received at least one dose of study medication (mITT set of patients) will be included in the safety analysis (treatment-emergent AEs). The incidence rate of the most pertinent and frequent ADR (> 5%) of each treatment group will be compared and tested. No adjustment for multiplicity will be performed. These results will be used for assessing the benefit risk ratio.

The proportion of patients with SAE and/or AEs leading to treatment discontinuation will be described per System Organ Class (using preferred terms defined by MedDRA), and summarised according to relationship to treatment and severity (CTCAE, version 4.03). In addition, a narrative for each of the SAE and AE leading to treatment discontinuation will be developed describing all aspects of the medical event.

The proportion of patients presenting at least one treatment emergent AE (TEAE) will be presented. All TEAEs will be described per study arm using the same classification as presented above. AEs will be tabulated by severity (CTCAE V4.03), and causality.

Abnormalities of laboratory/procedures results will be tabulated with respect to clinical significance. In addition, shift tables and graphs will be generated to describe the laboratory parameter values over time, per treatment arm.

10.8. PK Analysis

Initially, a standard two-stage non-compartmental analysis will be performed using the ncappc script in R to derive the following basic descriptive PK parameters for MF and PM in the intensive PK cohort: Cmax, tmax, $AUC_{0\text{-last time-point}}$, $AUC_{0\text{-end of treatment}}$ (MF). Groups of children ≤ 12 years and patients >12 years will be compared with each other. All results will be descriptively compared to historic data of published MF PK and unpublished PM PK data from the region. Subsequently, in a later phase, a population PK approach will be employed to derive better estimates of drug exposure and also to obtain standard primary PK parameters using a structural compartmental model. Using this model, a more sophisticated covariate analysis will be performed to assess the influence of combining both drugs on each other's PK parameters and additionally other time-constant and time-varying patient characteristics. Also sparse MF PK samples from all patients (in contrast to only the intensive cohort) will be included as well in such a population PK analysis. Exact details and procedures of this population PK analysis will be described in a population PK analysis plan. Such a structural population PK model will also allow for better assessment of exposure-response relationships, which will focus on linking individual exposure parameters to initial cure, final cure/relapse and data on parasite levels (both microscopy and qPCR) and potentially other measured markers of clinical improvement in patients.

10.9. PD Analysis

In this trial the blood parasite counts will be evaluated as a pharmacodynamic indicator to derive the systemic parasite clearance rate and thus as an experimental measure of response to treatment. Individual or pooled average (depending on the individual quality of data) parasite clearance rates will be derived using log-linear regression on positive parasite counts. This rate will be correlated to treatment outcome to evaluate the predictive value of this marker for the initial and final treatment outcome. Subsequently, using the developed population PK models, these will be linked in a sequential manner to the pharmacodynamic parasite counts. By following parasite clearance using qRT-PCR and evaluating PK of MF and PM, population PK-PD modeling will enable us to further explore the relationship between drug exposure and parasitological outcome in a more mechanistic way, which can be seen as an essential component in the development of new treatment regimens. Clinical outcome and measures (spleen and liver size), appropriate biochemistry values and other selected biomarkers will also be integrated as possible influential covariates in the PK-PD model, as appropriate. Eventually, the population PK-PD model will be used to identify which PK parameter and/or measure of exposure is critically relating to therapeutic response for MF and PM, which will be of pivotal value in defining the optimal treatment duration and intensity of MF in future combination regimens.

11. Data Safety Monitoring Board

A Data Safety Monitoring Board (DSMB), composed of a minimum of 3 members including a statistician and a physician all of whom must be independent of the investigator and sponsors, will be set up prior to study initiation. The DSMB will monitor the study in order to ensure that harm is minimized and benefits maximized for the study subjects. All safety and available outcome data will be presented to the DSMB for review. The data and intervals will be agreed prior to the study initiation and documented in the DSMB Charter.

12. Quality Assurance and Quality Control Procedures

12.1. Investigator's file

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents include Investigator's Site File, subject clinical source documents and screening / enrolment logs. The Investigator's Site File will contain the protocol/protocol amendments, CRF/SAE and query forms, IEC and regulatory approval with correspondence, sample informed consent, drug accountability records, staff curriculum vitae and authorization forms and other appropriate documents/correspondence etc.

12.2. Case report forms (CRFs)

Data will be collected by laboratory technicians, medical doctors, clinical officers and nurses authorized by the investigator. It will be supervised by the Investigator and signed by the investigator or by an authorised staff member. Study-specific information will be entered into the Case Report Form (CRF) and SAE form (if applicable). Data

that are derived should be consistent with the source documents or the discrepancies should be explained. All CRF data should be anonymised, i.e. identified by study patient number only.

The investigator at each trial site should ensure the accuracy, completeness, legibility, and timelines of all data reported to the sponsor in the CRFs/SAE forms and any other additional information that is required. The investigator is responsible for keeping all consent forms, screening forms, CRF/SAE form and the completed subject identification code list in a secure location.

12.3. Source documents

The verification of the CRF/SAE form data must be by direct inspection of source documents. Source documents include subject hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory reports, ECG, X-ray, pathology and special assessment reports, signed informed consent forms, consultant letters, and subject screening and enrolment logs.

The investigator must maintain source documents such as laboratory and consultation reports, history and physical examination reports, etc., for possible review and/or audit by DNDi and/or Regulatory Authorities. The Investigator / designee will record the date of each subject's visit in patient file together with a summary of their status and progress in the study.

12.4. Record Retention

The investigator must keep all study documents on file for at least 25 years after completion or discontinuation of the study. After that period of time the documents may be destroyed with prior permission from DNDi, subject to local regulations.

Should the investigator wish to assign the study records to another party or move them to another location, DNDi must be notified in advance.

12.5. Monitoring, audits and inspections

Monitoring visits to the trial site will be made periodically by DNDi representatives or designated clinical monitors to ensure that GCPs and all aspects of the protocol are followed. Source documents and SAE forms/Data clarification forms (DCFs; sent to DNDi pharmacovigilance) will be reviewed for verification of consistency with data on CRFs. The investigator will ensure direct access to source documents by DNDi or designated representatives. It is important that the investigators and their relevant personnel are available during the monitoring visits.

The investigators will permit representatives of DNDi and/or designated clinical monitors to inspect all CRFs/SAE forms/DCFs, medical records, laboratory work sheets and to assess the status of drug storage, dispensing and retrieval at any time during the study. The corresponding source documents for each subject will be made available provided that subject confidentiality is maintained in accord with local regulations. The inspections are for the purpose of verifying the adherence to the protocol and to ensure the study is conducted according to GCP. It is important that the investigators and other trial site staff are available at these visits.

The monitoring visits provide DNDi with the opportunity to evaluate the progress of the study, verify the accuracy and completeness of CRFs/SAE forms/DCFs, resolve any inconsistencies in the study records, as well as to ensure that all protocol requirements, applicable regulations, and investigator's obligations are being fulfilled. Four visit types are planned: pre-study, study start, during the study, and study end. Visits may also be performed by regulatory authorities.

It will be the clinical monitor’s responsibility to inspect the CRFs/SAE forms/DCFs at regular intervals throughout the study, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them. The investigator agrees to cooperate with the clinical monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

12.6. Audits and inspections

The trial site may also be subject to quality assurance audits by DNDi or designated representatives and/or to inspection by regulatory authorities or Independent Ethics Committees (IEC).

It is important that the investigators and their relevant personnel are available for possible audits or inspections.

12.7. Data Management

In order to ensure data quality, a uniform hard copy CRF will be designed for use at all the sites. After the CRFs have been completed by the site investigators and monitored by the clinical monitor, they will be collected and sent on an ongoing basis to DNDi data centre, based in Nairobi for data entry (double independent data entry), cleaning and analysis.

CRFs and source documents will be monitored by the clinical monitor. Discrepancies noted either by the monitor or data centre will be queried to the site PI. The trial data will be stored in a computer database maintaining confidentiality in accordance with national data legislation and Good Clinical Practice.

12.8. Confidentiality of trial documents and subjects records

The investigator must assure that subjects’ anonymity will be maintained and that their identities are protected from unauthorized parties. On CRFs or other documents submitted to the sponsor, subjects should not be identified by their names, but exclusively by an identification code. The investigator should keep a subject enrolment list showing codes, names, and addresses. The investigator should maintain documents for submission to sponsor authorized representative, and subject’s signed written consent forms, in strict confidence.

13. Protocol Amendments

The Principal investigator will ensure that the study protocol is strictly adhered to throughout, and that all data are collected and recorded correctly on the CRF. The Principal investigator may contact the medical coordinator for a protocol waiver for minor deviations from the protocol, e.g. patient unable to attend during visit window.

All protocol modifications must be documented in writing. Any protocol amendment must be approved and signed by the sponsor and the Principal investigator and is to be submitted to the appropriate IEC for information and approval in accordance with local requirements, and to regulatory agencies if required. Approval by IEC (and Regulatory Authority, if applicable) must be awaited before any changes can be implemented, except for changes necessary to eliminate an immediate hazard to trial subjects, or when the change involves only logistical or administrative aspects of the trial (e.g. change in clinical monitor(s), change of telephone number(s)).

The protocol amendment can be initiated by either sponsor or by any Principal investigator.

The investigator will provide in writing the reasons for the proposed amendment and will discuss with the medical coordinator and sponsor.

14. Early Termination of the Study

Both the sponsor and the investigator reserve the right to terminate the study at any time prior to inclusion of the intended number of subjects, but they intend to exercise this right only for valid scientific or administrative reasons. Should this be necessary, both parties will arrange the procedures on an individual study basis after review and consultation. In terminating the study, the sponsor and the investigator will assure that adequate consideration is given to the protection of the subject's interest.

Reasons for early termination by the sponsor(s) may include but not be limited to:

- Too low enrolment rate.
- Protocol violations.
- Inaccurate or incomplete data.
- Unsafe or unethical practices.
- Questionable safety of the test article.
- Suspected lack of efficacy of the test article.
- Following the recommendation of the DSMB or IEC
- Administrative decision.

Reasons for early termination by the investigator may be:

- Insufficient time or resource to conduct the study
- Lack of eligible patients

In the event that a study is early terminated either by the sponsor or by the investigator, the investigator has to:

- Complete all CRFs to the greater extent possible

- Return all test articles, CRF, and related study materials to the sponsor who provided them
- Answer all questions of the sponsors or their representatives related to data of subjects enrolled at the site prior to study termination
- Ensure that subjects enrolled in the study who had not yet reached a follow up time point are followed up with the necessary medical care.
- Provide in writing the reasons for his decision to the national health authority and the sponsor.

15. Ethics

The experimental protocol for this study has been designed in accordance with the general ethical principles outlined in the Declaration of Helsinki and ICH guidelines for Good Clinical Practice (International Committee for Harmonization). DNDi assures that it will comply with all applicable state, local and foreign laws for protecting the rights and welfare of human subjects. This protocol and any protocol amendments will be reviewed / approved by an IEC before its implementation.

It is the responsibility of the Global or National Coordinating Investigator/Investigator to apply for review to the IEC of the country where the study takes place regarding local rules and regulations. Written approval from all involved IECs must be obtained before implementation of any protocol-specified intervention /investigation provided to the subject (such as subject information sheets or descriptions of the study).

Any modifications made to the protocol after receipt of the IEC approval must also be submitted by the investigator in writing to the IEC in accordance with local procedures and regulatory requirements.

15.1. Informed consent process

Inclusion in the study will occur only if the subject (for adults) or the parent/guardian (for children) gives written informed consent. It is the responsibility of the investigator / designee to obtain written informed consent from each individual participating in this study, after adequate presentation of aims, methods, anticipated benefits, and potential hazards of the study. The written informed consent document will be translated into the local language or a language understood by the subject or the parent/guardian. If needed, the person will be given time to discuss the information received with members of the community or family before deciding to consent. The subject or parent/guardian will be asked to provide written and signed consent.

If the subject is illiterate or unable to write, a literate witness must sign (this person should have no connection to the research team and the sponsor, and, if possible, should be selected by the participant). The investigator should also obtain the assent of children (if appropriate and according to country regulatory requirements), but their assent must be completed by the permission of a parent or guardian.

Patients who agree to participate to the intensive PK and PD sampling (intensive cohort) will be asked to sign a separate informed consent document describing the

additional procedures to be performed.

In addition, depending on local country regulations, a separate consent form for sample storage will also be used as applicable.

If new safety information results in significant changes in the risk/benefit assessment, the consent form should be reviewed and updated if necessary. All subjects (including those already being treated) should be informed of the new information, given a copy of the revised form and give their consent to continue in the study.

15.2. Ethical aspects of subject inclusion and study procedures

Potential study subjects will be selected from the routine VL diagnosis and treatment activities at the study sites. Children are a majority of patients affected by VL in Eastern Africa. Following the routine procedures for diagnosis of Kala-azar, patients with a confirmed VL diagnosis, aged 4-50y will be invited to participate in the study. The limit of 50 years of age for inclusion is due to higher mortality rate and lower efficacy observed in patients >50 years when treated with SSG-PM in a recent pharmacovigilance program in Eastern Africa (Kimutai et al., 2017). This study showed a higher risk of death for patients aged ≥50 years (9.1%) versus the overall population (0.9%). The end of treatment effectiveness for this population was significantly lower (81.8%) as compared to the overall EOT effectiveness (95.1%).

The ICF process will be done with the subjects or parent/guardian of subjects in case of children.
Inclusion/exclusion criteria will be systematically assessed during screening phase and only those meeting the study criteria will be eligible for enrolment.

Parasitological confirmation of VL requires an invasive procedure, as tissue needs to be collected either from bone marrow or spleen. Tissue aspiration will only be performed by authorized medical personnel following the standard operation procedure. Patients with platelet count < 40,000/mm^3, Hb < 5g/dL or with signs of bleeding should be diagnosed by bone marrow rather than spleen aspirate.

HIV co-infected patients are excluded from the study, as they present lower efficacy rates, higher toxicity and higher lethality as compared to the non-HIV patients. Therefore, these patients require different case management and care.

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 will be excluded from the trial. Depending on countries and communities, it is very sensitive to introduce family planning to the young or unmarried female population who is not so familiar with contraception and is often illiterate. Community leaders' opinion will be requested to guide the trial staff in the best approach to open the discussion on this topic, taking into consideration the local cultural context from the different communities.

Study procedures are designed to ensure accurate assessment (i.e. clinical, PK sampling, laboratory assessments) while ensuring close follow up for safety. Patients in test arms 1 and 2 will be treated as in-patients up to day 14 to closely monitor the treatment safety and response to treatment. Patients in arm 2 used to continue on an out-patient basis until completion of the 28 days treatment. Since MF safety profile has

been studied in the past, no major safety concerns were expected during this period. In case of any safety concern, patients were instructed to either contact the investigator on phone or to come to the hospital for an unscheduled visit. Both patients in arm 1 and 2 are assessed at the end of treatment visit on day 28.

Furthermore, in order to record long-term clinical outcome, patients will be requested to return for the 6 months follow-up visit.

Biological samples will be drawn only for the purpose of clinical assessments, pharmacokinetic (PK) and pharmacodynamics (PD) analysis. The volume of blood collected will be minimised as much as possible, and only one venepuncture will be performed per planned scheduled visits.

Blood will be collected for PK analysis only in a subset of patients. Provided the PK nonlinearities observed in pediatric patients receiving the allometric MF dosing (LEAP0714), the inclusion of children ≤12 years old in the PK sub-study in addition to adolescents/adults >12 to ≦50 years is important to better study MF exposure in this population. 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.

A mixed sampling approach will be performed in order to reduce as much as possible the number of total samples and volume of blood to be collected (see section 8.4).

It is expected that the maximum total volume of blood to be collected in the intensive PK/PD cohort (subset of patients, 40 in arm 1 and probably less than 40 in arm 2 that is now discontinued) will be approximately 58mL over the 6 months participation in the trial, assuming:

6mL will be collected at study visits: screening, D7 and D28

12mL will be collected at D1

4mL will be collected at D3, D56 and D210

14mL will be collected at D14

2mL will be collected at D21 (in arm 2 only, not applicable in this protocol version).

Patients not included in the intensive PK/PD cohort will have a total of 32 ml of whole blood collected over the entire study, for blood safety assessments, MF PK and for qPCR.

There may be slight variations in the blood volume due to tubes available in the market. In any case, the maximum volume of blood to be collected in a study visit will be 14mL.

Biological samples may be kept after the completion of the study in order to evaluate how other biomarkers or immunological markers respond following VL treatment.

15.3. Ethical aspects of study treatments

MF is a drug marketed for Visceral Leishmaniasis indication and it has been

extensively used as primary treatment for VL in Asia, with very good safety profile. PM has been well studied in Eastern Africa during the development of the combination SSG-PM, which is now the current WHO-recommended treatment for VL. Safety profiles of these drug are well-known and potential side effects will be monitored throughout study duration to guarantee patient's safety.

All screened but ineligible VL patients will be treated with current standard treatment according to national guidelines.

15.4. HIV status and VCT

All patients will be offered counselling and screening for HIV (voluntary counselling and testing programme (VCT). This may either be done at the same time as consent is obtained for inclusion in the trial or at a later date according to hospital practice.

Patients who are found to be HIV positive will not be eligible to participate in this trial but will receive appropriate treatment, according to national treatment guidelines. Additionally, they will be referred onwards for treatment, surveillance and follow up according to the national protocol for HIV positive patients.

15.5. Patient costs

Patients will be reimbursed for travel to and from the study site but will not receive any payment for trial participation. Any medication that is required during the trial period will be provided free of charge to the patient. Food during the in-patient treatment phase will also be provided free of charge to the patient. This is seen as an essential part of the patient care plan bearing in mind the high prevalence of malnutrition and the poverty of these patients.

16. Insurance and Liability

DNDi is insured to indemnify the investigator against any claim for damages brought by a research subject who suffers from a research related injury during the performance of the trial according to the protocol. The insurance in each country will also cover for the costs of medical care required as a result of the subject participation in the trial (ex. AE related to study drug or study procedure).

17. Reporting and publication

All clinical trials will be registered with a recognised clinical trial registry such as www.clinicaltrials.gov. Once the trial is completed and published, open access policy for data sharing will be followed.

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