PROTOCOL SYNOPSIS

Protocol Title	An Open label, Randomized, Clinical Trial of Two Regimens to Assess the Safety and Efficacy for Treatment of PKDL Patients in the Indian subcontinent
Phase	Phase II
Indication	PKDL patients in India and Bangladesh
Protocol Number	DNDi-MILT/COMB-01-PKDL
Background Information and Trial Rationale	Post-Kala-Azar Dermal Leishmaniasis (PKDL) commonly follows visceral leishmaniasis (VL) caused by *L. donovani*. It is characterized by skin lesions in which parasites can be identified, in a patient who is otherwise fully recovered from VL or exposed to *L. donovani* infection. It occurs in the Indian subcontinent (mainly India and Bangladesh) as well as in Africa (mainly Sudan), where *L. donovani* is the causative parasite. There are major differences in epidemiology, clinical presentation and treatment between these regions. The reasons for these differences are not well understood. Factors related to the parasite and the host may play a role. **<u>Epidemiology</u>** In Bangladesh, VL is most common in Fulbaria and Trishal sub-district of district Mymensingh. In a cross-sectional study an increase in estimated PKDL incidence was observed from 1 case to 21 cases per 10,000 person-years in 2002-2004 and 2007, respectively. In another cross-sectional study (2007) the point prevalence of PKDL was estimated in 2007 at 3.8 – 7.3/10,000, with PKDL rates of 13.9-16% among patients who had a VL episode; 60 % of patients presented PKDL within 24 months, with 20% presenting within 6 months after VL treatment. In a series of cross-sectional surveys between 2002 - 2010, the cumulative incidence of PKDL was estimated at 17% by 5 years. Most patients had been treated with SSG (95%); the mean interval between VL and PKDL was 19 months. In Trishal sub-district, in a cross-sectional study, the prevalence of probable PKDL was 6.2/10,000 people in 2010. The median time between VL and PKDL was 36 months (IQR, 24-48 months). All had been treated for VL with Sodium Stibogluconate (SSG).

	A recent longitudinal study in Fulbaria (2015) of 1918 VL patients with active follow-up showed PKDL rates of 10.2% at 36 months (mean 17.6 months; range 4-43 months) after VL treated with 3 x 5 mg/kg Ambisome®. In all above studies, diagnosis was based on clinical judgement and not based on microscopy or PCR report. In India the only recent cross-sectional study from 2012 estimates the prevalence of PKDL in 16 VL-endemic communities in Bihar as 4.4/10,000 for confirmed cases and 7.8/10,000 when probable cases were included. Cases had been treated for VL with SSG, paromomycin or amphotericin B. Also in Bihar, in an observational, retrospective cohort study with passive case-finding of PKDL in 8311 VL patients treated for VL with Ambisome® 20 mg/kg, PKDL was diagnosed in 0.3%. The interval after VL was longer in a small group treated with SSG (2.9 years in the SSG group vs. 1.2 years for the Ambisome® group). The interval between VL and onset of PKDL was 1.5 ±0.9 years (range 0.5-3.4); the interval between VL and PKDL diagnosis was longer: 2.4 years (range 1.0-4.4). In Africa, PKDL is even more common but there are important intraregional differences. In Sudan it is most common: up to 50-60% of VL cases develop PKDL, usually within 6 months after VL episode, and virtually all cases develop within 12 months (mean 4.5 months). In Ethiopia, Kenya and Uganda PKDL is less common for reasons that are not well understood. Factors relating to the host, the parasite and treatment given for VL may all play a role. PKDL in patients co-infected with HIV is more common and more severe; in Ethiopia in areas where 40% of VL patients are HIV infected, PKDL is more common (28% vs. 14%, in HIV positive and HIV negative patients, respectively) and more severe. PKDL is also occasionally seen in VL caused by *L. infantum* in particular in HIV-VL co-infected patients. **<u>Clinical presentation</u>** The clinical presentation varies in each region: in Bangladesh >90% of patients have a macular rash. While the face is often involved, macules may occur on all parts of the body, without a clear pattern. In India the rash is often polymorphic with a mixture of macules, papules and nodules. It should be noted that most reported cases are from tertiary referral centres and may reflect chronic PKDL cases of long duration, thus presenting with more advanced disease. The PKDL patients do not have other associated signs or symptoms, and apart from the skin lesions they are generally healthy individuals. In Africa (Sudan) the rash is usually papular or maculopapular; in severe cases these coalesce to form nodules or plaques. A minority of patients present with a macular rash only. The rash usually affects the face and may spread to arms and trunk and further caudally to involve the whole body in severe cases. The clinical distribution is thus described in 3 grades of severity taking

	into account the degree of spread. **Pathogenesis and natural history** PKDL often occurs in the sun-exposed parts of the body suggesting the influence of UV light. It has been shown that PKDL occurs when the immune response shifts from a Th2 response in active VL to a mixed Th1 / Th2 response after treatment for VL with persistence of IL-10. This means that while systemically there is a predominantly Th1 response as a result of VL treatment and cure (i.e. with systemic clearance of parasites), in the skin a Th2 response persists with demonstrable parasites which leads to the clinical presentation described above. There is little information on the natural history of PKDL in Bangladesh and India. While apparently the interval between VL and PKDL is documented as long (median 2 years), there is limited information that this may be shorter when patients are actively followed after VL. Indeed, some PKDL cases may present during VL or shortly after VL treatment. Self-healing has been described in Bangladesh in two reports by retrospective history (self-reported by the patient, 8% resolution after 1-year) and a few early cases not well characterized. This finding has not been confirmed by direct observation of patients. In practice, in Asia, as most patients are diagnosed late, it is assumed all patients are chronic and self-healing is not expected. All PKDL cases are treated, irrespective of the extent of the rash. Studies from Sudan show that most PKDL patients (85%) self-heal within 12 months, majority within 6 months of PKDL onset. Only chronic patients are treated and those who present with severe disease (grade 3). Skin smears and biopsies from lesions may demonstrate the presence of parasites and it is thought that PKDL patients play a role in transmission (anthroponotic cycle) as (limited) studies have found that sand flies feeding from PKDL patients become infected with Leishmania parasites. There is therefore an important public health concern that these patients may, for the duration of their rash, be infectious to sand flies and thus play a role in transmission. **Treatment** In the Indian Subcontinent, all patients are treated. In a cohort study of 26 patients treated with miltefosine (2011), 23 of 24 patients who completed the study were cured (96%), with no relapses after 12-months follow-up. Of these, 13 received miltefosine 150mg (50mg tid) for 60 days, 3 patients received miltefosine 150mg (50mg tid) for 75-90 days, and other 7 patients had the dose reduced to 100 mg daily (50mg bid) due to gastrointestinal complaints, and were treated for 90 days. The one patient who failed had received miltefosine 150mg (50mg tid) for 90 days. In another study from India (2013), miltefosine 100 mg/day for either 12 or 8 weeks was evaluated. The ITT and per-protocol cure rates after 12 months of

	follow-up for patients receiving 12 weeks were 78% (14/18 patients, 95% CI = 61–88%) and 93% (14/15 patients, 95% CI = 71–95%), respectively, whilst the ITT and per-protocol cure rates for patients receiving 8 weeks were 76% (13/17 patients, 95% CI = 53–90%) and 81% (13/16 patients, 95% CI = 57–93%), respectively. In a study from India (2015) of 33 patients treated with miltefosine 100 mg (50mg bid) for 12 weeks, the cure rate was 97% (28/29 patients that could be evaluated) after 1-year follow-up. In 1 patient treatment was stopped because of adverse effect. It should be noted that in a recent descriptive study (2015) from India (non-randomized, non-controlled, unblinded observational study), decline of clinical efficacy of miltefosine was reported; 11/73 (15%) of PKDL patients relapsed by 18-months follow-up and this was highest (31%) in those with shorter duration treatment (50 mg tid for 60 days) as compared with longer duration (50 mg bid for 90 days) of which 10.5% relapsed. Parasite load at baseline was significantly higher in relapse patients as compared to cured cases. Previously similar decrease in response to miltefosine in treatment of VL has been reported. Treatment of 60 PKDL cases in India with SSG 10 mg/kg for 90 days, resulted in improvement with disappearance of parasites but the difficulty in assessing cure was emphasized, in particular in macular lesions. In imprint smears, the mononuclear infiltrate decreased (papular lesions) or persisted (macular lesions). In a recent field experience by MSF on routine patient management in Bangladesh (Fulbaria), 1400 patients were treated with Ambisome® 6 x 5 mg/kg given over 2 weeks. Results were satisfactory, with 86.5% of patients presenting total disappearance of all nodular and papular lesions and complete or very significant re-pigmentation of macular lesions after one year of follow-up. Among the 13.5% of cases who did not have significant re-pigmentation of macular lesions at 12 months, improvement was observed in subsequent visits, and only 2% of the patients required a second course of treatment. This experience was based on clinical assessment in the field, and not done systematically by standardized procedures. In order to better characterize the Ambisome treatment for PKDL patients in Bangladesh, MSF conducted a clinical trial where patients were again treated with Ambisome® 30mg/kg (n=110). In this trial, treatment outcome was standardised, including photographs and evaluated independently by clinicians. A total of 88 patients completed 12-month follow up, out of which 80% had satisfactory response (67% patients had complete resolution of lesions and 13% had substantial improvement (>75%) of lesions). In this study however, severe side-effects were noted as rhabdomyolysis was reported in 6 patients, 3 of which had severe hypokalemia that was ascribed to Ambisome® therapy. In an on-going second study, patients are treated with 5 x 3 mg/kg (2 injections per week) with potassium and magnesium supplementation and intensive monitoring. Preliminary reports do not suggest severe hypokalemia occurring

	during this regimen, and patients are responding to treatment satisfactorily. In addition, expert opinion in Bangladesh is that patients with severe PKDL in Bangladesh had to be treated with high total doses of Ambisome (up to 60mg/Kg total dose) and careful monitoring of treatment help to prevent severe hypokalemia or rhabdomyolisis (Dr. Mondal, personal communication). Perhaps severe PKDL leads to significant destruction of the skin and this in addition to higher dose of AmBisome facilitate better penetration of the drug to the skin. This emphasizes the need of skin PK analysis of anti-PKDL drugs and to correlate it with response to treatment. In Africa (Sudan) only chronic PKDL patients, i.e. showing persistence of lesions after 6 months of disease onset, and those with severe PKDL are treated. There are no conclusive studies on treatment of PKDL in Sudan; current practice is treatment with SSG 20 mg/kg for 40-60 days. There is limited experience with Ambisome®, one study showed cure rate of 83% (10/12 patients) with Ambisome® given intravenously at 2.5 mg/kg per day for 20 days, without detectable adverse effects. Recent data showed good cure rates of combined SSG 20 mg/kg and paromomycin 11 mg/kg for 30-40 days in 8 of 9 patients treated. As the pathogenesis of PKDL implies an important role for the immune response, there is a rationale for immune modulation. In one study, combined immunochemotherapy with alum-precipitated autoclaved Leishmania major (Alum/ALM) vaccine + Bacille Calmette-Guérin (BCG) and SSG showed better cure rates than SSG alone (87% vs. 53%). Newer immunomodulatory agents are under development but are not expected to enter clinical studies until 2018. Currently there are no immune markers that may guide the duration of treatment or indicate outcome. **<u>Rationale for the study and selection of treatments to be tested</u>** In the Indian Sub-continent (ISC) all patients with PKDL are treated irrespective of interval after VL, previous VL treatment, duration of the rash, or clinical presentation. While treatment may be indicated for clinical reasons, it is thought that PKDL patients may play an important role in transmission of VL and chronic PKDL patients have been implicated in major VL outbreaks in the past. Obviously, this is of crucial importance in the current VL elimination efforts in the ISC. This study aims primarily to improve current treatment options. In addition, this will be the first study ever in PKDL in which outcome will be described in clinical, parasitological and immunological terms in relation to pharmacokinetics thus providing the rationale and focus for subsequent studies. Currently there are no satisfactory treatments for any forms of PKDL. Available treatments include pentavalent antimonials: sodium stibogluconate (Pentostam, Stibonate) and meglumine antimoniate (Glucantime) which have been used since the 1940s and in some regions,

	such as Sudan remain as the main first-line drugs. Conventional amphotericin B has been used in India for prolonged periods (60 infusions) but this is impractical and requires careful clinical and biochemical monitoring. Both miltefosine and Ambisome® as monotherapy have shown to be effective. However, with the current recommended schemes there are some drawbacks such as the length of the treatment with miltefosine alone (8-12 weeks); the costs and recently observed toxicity of a high dose Ambisome® (total dose of 30 mg/kg). There is also the potential risk for development of resistance with miltefosine as monotherapy. In addition, there is an issue of access, as currently there is only one manufacturer for MF and situations of drug stock rupture have already occurred at some occasions, leaving no drugs in filed for treatment of PKDL patients. The current scenario does not offer many alternatives given that no new drugs are available or in late stage of development. Miltefosine, despite its teratogenic problems, is currently the only oral drug available with the advantages that this route of administration offers. Rather than dismissing the use of miltefosine due to its teratogenic effects, efforts to optimize its use by combining it with other drugs aiming to provide a safe, efficacious and shorter treatment with a high compliance should be explored. In terms of numbers, PKDL cases occur less frequently compared to VL cases which makes it possible to monitor contraception properly; indeed, no large scale PKDL treatment programs are expected and treatment is focussed on individual PKDL patients. Combination treatment is well accepted in the treatment of VL, paromomycin (PM) combined with miltefosine in Asia; or SSG combined with PM for example in East Africa and may offer shorter duration of treatment thus preventing prolonged hospitalization. Another possible advantage is prevention of development of resistance and reduced cost. These principles also apply to PKDL, where the need for an ambulatory treatment with a highly safe, efficacious and user-friendly regimen is even more pressing as patients are not ill, except for the rash. While ideally the choice of study regimens would be based on drug-penetration of PK levels in the skin and the plasma, this would require studying a large number of patients with drugs in regimens that may or may not be appropriate as future PKDL treatment in the ISC. Therefore, we have chosen to design a study with 2 treatment arms that reasonably could be effective and safe for PKDL treatment and integrate the PK study so as to guide future treatment studies also depending on outcome. Except for the presence of localized skin lesions, subjects with PKDL are otherwise healthy, so we may assume that the pharmacokinetics of Ambisome® and miltefosine in these subjects could be similar to that in healthy volunteers. However, there is no information about the levels of Ambisome® and miltefosine in skin of human or animal studies and how it correlates with plasma concentrations. This study aims to determine if accumulated Ambisome® and miltefosine levels are detectable in the skin of

	PKDL patients at the end of treatment, and if so how these skin levels relate to the individual systemic concentrations of the drugs and their correlation to the in vitro drug susceptibility of the Leishmania parasites. Miltefosine concentrations keep accumulating until the end of the 28-day treatment period in patients. Therefore, we expect that a single tissue sample at the end of treatment will indicate the extent of miltefosine accumulation in skin tissue, even when assuming a delayed tissue distribution. In contrast to the fungal diseases, in which the free amphotericin B fraction probably represents the active component of Ambisome®, in leishmaniasis it is most probably the liposomal fraction of the drug, as this is the fraction that is preferably phagocytosed by the host's macrophages in which the Leishmania parasites reside. The liposomal fraction of Ambisome® also represents the largest fraction of the drug in circulation after administration in human. Given the fact that logistic difficulties will preclude us from differentiating between free, tissue-bound, protein-bound and liposomal amphotericin B in this field trial, total amphotericin B levels will be measured in the skin and plasma and used for pharmacokinetic assessments. This exploratory study proposes to determine the efficacy and safety of Ambisome® monotherapy regimen (5 x 4 mg/kg, given twice per week, total dose of 20 mg/kg) and Ambisome® (5 x 4 mg/kg, given twice per week, total dose of 20 mg/kg) in combination with miltefosine daily for three weeks (allometric dosing), for PKDL patients in the ISC. The limitation of Ambisome® at the total dose of 20mg/Kg is due to the safety concerns, mainly the cases of rhabdomyolysis observed in Bangladesh with the dose of 30mg/Kg. It is assumed that by decreasing the dose to 20mg/Kg safety will be improved with no significant loss of efficacy. The Ambisome® will be given in 5 administrations over 2 ½ weeks to reduce safety risks. In addition, PD data from previous DNDi trials on VL have shown that parasite clearance is faster when Ambisome® was administered in multiple injections as compared to single administration. Although in this protocol we are not evaluating VL, but PKDL, splitting the dose over time may be beneficial for parasite dynamics, which will be monitored over time. Miltefosine has a long half-life (7 days) and accumulates to eventually reach a steady state after 4 weeks of treatment. A minimum of 3-weeks treatment was considered necessary to reach sufficient exposure for the activity of miltefosine in PKDL patients. Still, this is a significant reduction as compared to the current 8-12 weeks of monotherapy regimen. If the proposed scheme shows to be safe and efficacious, it may be possible to consider regimens to treat PKDL which have shorter duration of treatment thus increasing compliance. It has long been known that the underlying mechanism for controlling Leishmania infection and self-healing of dermal lesions is the cellular immune response, mediated by antigen-specific γ-interferon producing T-cells. Additional information to better understand the status and role of the host

	immune response and how it interacts when different treatment modalities are provided is needed. Similarly, studies to demonstrate that appropriate concentration of the drug is present in the right compartment, and its correlation with the clinical outcome are crucial when optimizing a treatment modality. Lastly, data on parasite clearance during treatment of PKDL and follow-up may be correlated to immunological parameters and clinical outcome and thus may contribute to identify the best parameter to indicate cure in PKDL.
Trial Objectives	General Objectives: The overall objective of this study is to assess the safety and efficacy of two treatment modalities for PKDL patients in ISC. **Primary Objective:** To measure the safety and efficacy of Ambisome® monotherapy regimen (5 x 4 mg/kg IV, twice per week, total dose of 20 mg/kg) and Ambisome® (5 x 4 mg/kg IV, twice per week, total dose of 20 mg/kg) in combination with miltefosine allometric dose orally for three weeks, for PKDL patients in the ISC. **Secondary Objectives:** • To assess skin and plasma concentrations of total Ambisome® and miltefosine. • To evaluate the host immune response in each treatment arm before, during and after treatment. • To evaluate parasite clearance in each arm as indicated by direct microscopy and qPCR • To assess relationship between clinical, parasitological and immunological responses to identify a potential biomarker for cure. • To assess relationship between pharmacokinetic parameters with clinical outcome and parasite clearance. **Exploratory Objective:** • To assess non-invasive tape disc method of skin sample collection for molecular parasitological diagnosis.

Trial Endpoints	**<u>Primary Endpoints:</u>** Efficacy To measure the efficacy (definitive cure at 12 months) of two treatment regimens in subjects with PKDL according to clinical criteria: complete resolution of papular and nodular lesions (flattening of 100% of lesions) and significant improvement (> 80% re-pigmentation) of macular lesions by 12 months after the end of treatment. Safety: To assess the safety of the two regimens (SAEs. AESI and frequency and severity of AEs) from the start of treatment through the 12-month follow-up period and 24 months study visit. **<u>Secondary Endpoints:</u>** Efficacy • To measure the efficacy of the two treatment regimens in subjects with PKDL according to clinical criteria: complete resolution of papular and nodular lesions (flattening of 100% of lesions) and significant improvement (> 80% re-pigmentation) of macular lesions by 24 months after the end of treatment. • To assess the overall assessment of the clinical presentation of PKDL lesions by the investigator at 12 and at 24 months according to the following categories: • Category 1: All lesions have improved, and the majority of lesions have resolved. Any remaining lesions are only faintly visible as re-pigmentation is almost complete. • Category 2: The majority of lesions show significant improvement some lesions have resolved. • Category 3: No new lesions, but the majority of lesions show no or slight improvement. • Category 4: New lesions have appeared and there is no or little improvement of other lesions Pharmacokinetics • To assess the maximal accumulation of total amphotericin B and miltefosine in the skin at the end of treatment and correlate these with plasma levels. Immune Response • To assess the change in immune response during and after end of treatment as compared to baseline by measuring cytokines profiles level in the peripheral blood.

	Parasitology • To assess the clearance of parasites by microscopy and qPCR (samples collected from skin, blood and tape disc) at various time-points before, during and after treatment and during follow-up.
Trial Design	This is a non-comparative, open label, randomized phase II clinical trial to assess the safety and efficacy of Ambisome® monotherapy (5 x 4 mg/kg IV given twice per week at a total dose of 20 mg/kg given) and a combination of Ambisome® (5 x 4 mg/kg IV given twice per week at a total dose of 20 mg/kg) plus miltefosine allometric dose orally for three weeks for PKDL patients in the ISC. Strategies to reach PKDL patients will be adapted according to the site context. Activities may include active case detection, targeted active case detection based on VL registry at the site, referral network from dermatologists, etc. Subjects will be hospitalized during the administration of Ambisome®; although hospital-day care at the dates of Ambisome® injection will also be acceptable. The miltefosine treatment will start at the same time as Ambisome® treatment, and it will be continued until completion of 3 weeks on an out-patient basis. During hospitalization compliance to treatment is assured. For patients randomized to the combination arm, the patient or parent/guardian (in case of children) will be educated on the administration of miltefosine, and how to manage possible gastro-intestinal events. Patient is discharged with clear instructions on how to continue miltefosine treatment, and when to return for clinical assessment. Study assessments will be performed at baseline, D1, D8, D15, D22 (only for subjects allocated to combination arm), and D30 (+/- 3 days) during treatment phase. All subjects will have follow-up visits at 3 months (+/- 7 days), 6 months (+/- 7 days), 12 months (+/- 14 days), and 24 months (- 1 month/+ 3 months) after the onset of treatment to assess efficacy and safety. A strategy will be put in place according to the context of each trial site to assure patients comply with the schedule of scheduled study visits. This can include active tracking of the patients through telephone contact or domiciliary visits. At any time-point during the study, if the patient does not respond to treatment, or lesions are worsening, or if after the healing period, the lesions reappear/reactivate, rescue treatment can be initiated at the discretion of the study investigator. A subject who receive rescue treatment at any time during the trial will be considered a treatment failure and will be withdrawn from the study. **<u>Sample collection</u>** PK samples from the skin (biopsy) will be collected at the end of treatment i.e. at day 15 for patients allocated in the Ambisome® arm and day 22 for

	patients allocated in the Ambisome® + miltefosine arm. For patients allocated to Ambisome+miltefosine arm, miltefosine concentration will be measured in EDTA blood plasma collected at D8, D15, D22, D30 and 3 months visits. In addition, miltefosine concentration will be measured in the skin biopsy collected at D22 from patients allocated to combination arm. For a sub-sample of 30 patients per arm, total amphotericin B concentration will be measured in EDTA blood plasma collected at D1 (at the end of infusion, and at 2, 4, 8, and 22 hrs after the end of infusion), D15 (at prior to infusion, at the end of infusion and at 2, 6, and 22 hrs after the end of infusion) and at D22 (single sample for combination arm only) or D30 (single sample for monotherapy arm only). In addition, total amphotericin B concentration will be measured in the skin biopsy collected at D15 from patients allocated in the Ambisome monotherapy arm, and at D22 from patients allocated to combination arm. As much as possible, a single blood draw should be performed per study visit to avoid multiple vein punctures. Sample for PK will be a 2mL EDTA whole blood sample/time point. Blood samples for immunological studies will be collected at baseline, D15, D30 and at 3, and 12-months follow-up visits. Blood samples for qPCR will be collected at baseline, Day 30 (EOT) and at 3 and 12 months follow up visits. Skin snip samples for parasitological examination by microscopy and qPCR will be collected at day 0, D30 (EOT), 3-months, 12-months and 24 months follow-up. In addition, as an exploratory objective, skin sample will be collected at the same time-points, with the exception of 24 months, using non-invasive tape disc method, from where DNA will be extracted for qPCR. Miltefosine and total amphotericin B (free + protein-bound + liposomal) concentrations in plasma and skin biopsy samples will be determined using validated bioanalytical methods employing liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Parasite loads will be measured by quantitative real-time PCR (qRT-PCR) based on the detection of Leishmania kDNA target. Cytokines and cell count: IL-2, IFNY, TNFα, IL-10 and Granzyme B (and possibly other biomarkers, after a preliminary screening) will be measured in the stimulated whole blood supernatant by BD™ Cytometric Bead Array Human Th1/Th2 Cytokine CBA. Lymphocyte subsets will be identified by FACS analysis.
Main Entry Criteria Inclusion	Patients who fulfil all inclusion criteria and do not present any of the exclusion criteria will be eligible to be enrolled in this study.

Exclusion	**Inclusion criteria:** • Confirmed PKDL case by clinical presentation and demonstration of parasites by microscopy in a skin smear or biopsy or by PCR, with stable or progressive disease for at least 4 months • Male or Female patients aged 6 to 60 years • Written voluntarily informed consent from adult patient and from parent / guardian in case children <18 years old. In the case of minors, assent from the children will be obtained according to country regulations. **Exclusion criteria:** • Patients who had prior treatment of PKDL within last 2 years • Pregnant and lactating women and women of childbearing age (12 to 55 years) who, before randomization, cannot be assured contraceptive cover during treatment and 5 months thereafter • Patients with signs and symptoms of severe diseases: defined as suffering from a concomitant severe infection such as TB or any other serious known underlying disease (cardiac, renal, hepatic), • Severe malnutrition defined by BMI for age WHO reference curves for gender, Z score < -3 for subjects 6 - 19 years; BMI < 16 for subjects > 19 years old. • Patients with haemoglobin < 5g/dL • Patients with abnormal liver function (ALT and AST) tests of more than three times the normal range. • Patients with total bilirubin levels >1.5 times the upper normal range • Patients with serum creatinine above the upper normal range • Patients with serum potassium < 3.5 mmol/L • Patients with a positive HIV test, as applicable • Patients / guardian not willing to participate • Patients with history of allergy or hypersensitivity to the relevant study drug • Patients on immunomodulators

Study Duration	6 months are expected to be needed to obtain all ethical and official clearances. The recruitment period will be 12 months with possible extension up to 18 months depending on recruitment capacity and the follow-up period will be 24 months. Therefore, the overall trial duration will be 36-42 months (FPI to LPO).
Test Drugs	• Ambisome® monotherapy (5 x 4 mg/kg IV twice per week, for 2 ½ weeks, at D1, D4, D8, D11, D15) • Combination of Ambisome® (5 x 4 mg/kg IV, twice per week, for 2 ½ weeks, D1, D4, D8, D11, D15) plus miltefosine allometric dose orally BID administration for 3 weeks. An easy-to-use table with dosing scheme by weight, height, and gender, will be provided to the investigators to define the exact daily miltefosine dose to be administered.
Statistics	**Sample size** We estimated that a minimum sample size of 50 subjects per treatment arm would provide a precision estimate of 10% with 95% CI, based on an anticipated cure rate at 12 months in 85% of patients. Accounting for 10% subjects lost during follow up and 15% subjects with treatment discontinuation 13 more subjects were added per arm, resulting an increase of the sample size to 63 for each regimen. The overall sample size for the two regimens is 126 subjects. The anticipated cure rate was selected based on the criteria set on the cure rates previously reported with the use of Miltefosine or Ambisome® alone. A computer generated, randomization code will be used for patient treatment allocation to one of the two treatment arms indicated. Eligible patients who fulfil all the inclusion criteria and have none of the exclusion criteria, and from whom informed consent has been obtained, will be randomized to one of the treatment regimens. **Summary of analysis** Descriptive statistics will be used to present study data. Continuous variables will be presented as number of observations (n), mean, standard deviation (SD), median, minimum and maximum values. Categorical variables will be presented as counts and percentages. Median time to the development of any AE will be determined using life-table analysis. All data will be presented separately by treatment group, local and systemic AEs. AEs and medical history will be coded using the most recent version of the Medical Dictionary of Regulatory Activities (MedDRA) preferred terms and will be grouped by system, organ, and class (SOC)

	designation. Summary statistics will be provided by group including the frequency, severity, seriousness and relationship of AEs to study drug organized by preferred term by SOC grouping. Efficacy endpoints will be presented as summary statistics by treatment group and will be conducted once all subjects complete the 12-months study visit. The number of subjects who met the criteria for cure divided by the total number of subjects in that group will be presented by study group. In addition, secondary efficacy endpoints will be described for the 24 months follow-up. Subjects who are lost to follow-up will be compared to those completing the study with respect to study site, demographic, and clinical factors. If lost to follow-up exceeds 20% of the sample, attempts will be made to seek out these dropouts (or their proxies) to ascertain final outcomes. The attributes of people that are found through this process will be compared to those not lost to follow up. For the pharmacokinetic data analysis, a population-based approach will be used employing non-linear mixed effects modelling (using NONMEM), to take into account within- and between-subject variability in all pharmacokinetic parameters, to optimally identify potential covariates such as body size descriptors (age, lean body mass, total body weight, etc) and other demographic parameters, but also interaction effects such as treatment-arm related differences in pharmacokinetics parameter estimates. The objective function value (equals -2 log-likelihood, derived from NONMEM) will be used to assess statistical significance of evaluated covariates and hierarchical structural models. The individual blood plasma:skin distribution ratio will be estimated using the developed pharmacokinetic model. Individual blood plasma:skin ratios of drug concentrations will be contrasted with the IC90 values previously obtained for L. donovani parasites.