DNDi
Drugs for Neglected Diseases *initiative*

# CLINICAL TRIAL PROTOCOL

## Clinical Trial Protocol Title

**'Efficacy and safety of thermotherapy in combination with Miltefosine in comparison to miltefosine monotherapy for the treatment of New World Cutaneous leishmaniasis: A phase III, open label, multicenter, randomized trial'**

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| **Short title** | Combination and, Miltefosine Monotherapy for CL |
| **Name of product(s)** | Thermotherapy (ThermoMed™)<br>Miltefosine (Impavido®)<br>Meglumine Antimoniate (Glucantime®) |
| **Drug Class** | Phospholipid (alkylphosphocholine)<br>Antimonial |
| **Phase** | Phase III |
| **Indication** | Uncomplicated Cutaneous Leishmaniasis (CL) in the New World (NW) |
| **Clinical Trial Protocol Number** | DNDi-MILT-08-CL |
| **EudraCT** | Not applicable. |
| **Sponsor** | Drugs for Neglected Diseases *initiative* (DND*i*), Chemin Camille-Vidart, 15, 1202 GENEVA Switzerland<br>Phone: +41 22 906 9230 |

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| **Clinical Trial Protocol Version / Date** | FINAL v.1.0_09September2019 |
| **Protocol Amendment Number / Date** | Amendment 1 Protocol FINAL v2.0_09October2019<br>Amendment 2 Protocol FINAL v3.0_20May2020<br>Amendment 3 Protocol FINAL v4.0_22September2020 (for Peru ONLY)<br>Amendment 4 Protocol FINAL v5.0_19October2020 (for Peru ONLY)<br>Amendment 5 Protocol FINAL v6.0_13May2021<br>Amendment 6 Protocol Final v7.0_26April2022 (for Brazil ONLY)<br>Amendment 7 Protocol FINAL v8.0 13 July2022 |

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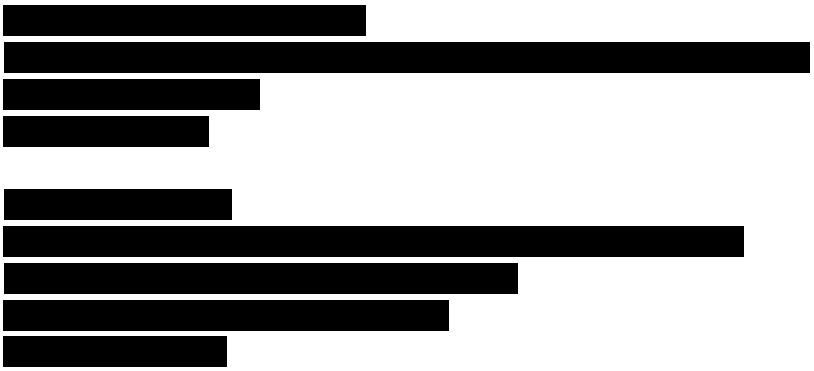

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## PROTOCOL SYNOPSIS

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| **Background Information and Trial Rationale** | Cutaneous Leishmaniasis (CL) is a parasitic disease caused by over 15 different species of the protozoan parasite *Leishmania.* CL typically begins as a papule at the site of a sand fly bite, enlarges to a nodule and ulcerates over 1–3 months. The exact incidence of CL is not known. An estimated 1.2 million cases/year from approximately 90 countries worldwide suffer from different forms of CL[1]. In general, most lesions become ulcerated during the course of the disease. Among the different parasites causing CL, *L. tropica* in the Old World and *L. braziliensis* in the New World (NW) are considered the most important because of the severity of the disease and the challenges of treating patients[2,3].<br><br>In 2016, 48.915 new CL and mucocutaneous leishmaniasis (MCL) cases were reported by 17 countries from Latin America (LA). It represents 5% more cases in comparison with 2015. 74.3% of all cases were reported from Brazil, Colombia, Nicaragua and Peru[4].<br><br>Pentavalent antimonials continue to be the first-choice drug despite their toxicity, difficulty in administration and high cost. Several randomized studies evaluating its efficacy in the NW, using the standard dose of 20 mg/kg/day/20 days parenterally, have shown a variable cure rate which goes from less than 50% up to 100% (Means: intent-to-treat (ITT) 70%, per protocol (PP) 77.4%,) (See table 1). Variations among different countries and species of *Leishmania* causing the diseases are common. In most LA countries antimonials, mainly meglumine antimoniate, are provided free of cost to all patients by the Ministry of Health, but shortage and limitation to properly provide the medication are common in many endemic districts[1,5,6].<br><br>Alternative treatment regimens include intralesional antimonials, miltefosine, pentamidine, isethionate, amphotericin B, antifungal agents (e.g., ketoconazole, fluconazole, itraconazole), paromomycin, and heat therapy or cryotherapy[1].<br><br>Following the WHO methodology for treatment guidelines development, in 2022, PAHO published an update version of their guidelines for the treatment of leishmaniasis in the Americas, which was developed, based on the evidence available in the region. The revised guidelines place miltefosine with a strong recommendation for the treatment of CL patients with lesions caused by *L. panamensis*, *L. guyanensis*, *L. mexicana* and *L. braziliensis,* including pediatric population[7], and also place with a strong recommendation the use of intralesional antimonial in adult patients with localized CL caused *by L. amazonensis and L. braziliensis.* The use of systemic antimonials was left only as conditional and in case there is no other options.<br><br>Miltefosine at the dose of 2.5mg/kg/day for 28 days orally was approved by the Food and Drug Administration (FDA) in 2014 for the treatment of CL in the New World for lesions due to *L. braziliensis*, *L. panamensis* and *L. guyanensis* only. Efficacy results from different studies in the NW are variable ranging from 49% to 94% (Means: ITT 70.2%, PP 78%) (see Table 2). The disparity in cure rates obtained in the different clinical trials is probably due to different geographical intrinsic sensitivity of *L. braziliensis* strains to miltefosine. In fact, it has been postulated that some strains of *L. braziliensis* may have a reduced capacity to internalize miltefosine from the extracellular medium[6]. A drawback of the use of miltefosine is that it must not be used in pregnant women, because of its known teratogenicity. Women of childbearing potential must receive effective contraception until 5 months after treatment.<br><br>Even though miltefosine is approved by FDA and listed by Pan American Health |

Organization (PAHO) as a treatment option for CL in the NW, it is barely used routinely by any CL national program due to the lack of registration in the countries and due to its costs. This situation may change in the near future since some drug companies are actively working to produce generic miltefosine. The assumption is that the generic miltefosine is going to be filed for World Health Organization (WHO) prequalification, which in turn may ensure the availability of a good quality manufactured drug, facilitating the registration/ use of the product in different countries and making it affordable by many national programs.

The Brazilian Ministry of Health has included miltefosine in their treatment recommendations as a first line treatment option.,. In this sense, in February 2020, Miltefosine was included in the Brazilian´s list of substances subject to special control (Resolution RDC 337, 11 Feb 2020) and has been deployed in the country since 2020[8,9]. Also, in Peru, miltefosine will soon be part of treatment recommendations for patients above 60 years of age and children who did not respond to antimonials, and other countries in the region have started reviewing their guidelines to adapt to new PAHO recommendations

The use of topical or local treatments for CL is an option that has been widely explored and it is currently one of the options listed by WHO in those situations where the patient has few lesions (≤ 4) of at most 4 cm in diameter and located in areas of the body which may be treated topically. The use of intralesional antimonials, liquid nitrogen or local heat (thermotherapy) are schemes recommended by WHO and regionally adapted by PAHO and WHO Eastern Mediterranean Regional Office (EMRO) for countries in the New World and Eastern Mediterranean region respectively. A Standard Operating Procedure (SOP) for preparing the lesions and applying local heat has been developed by EMRO[1, 10,11].

**Table 1: Efficacy of Antimonials reported from the New World when administered at 20 mg/kg/day/20 days parenterally to CL patients**

| Country | Leishmania specie | N | Cure rate (95% CI)* | Reference** |
|---|---|---|---|---|
| Belize | *L. braziliensis / L. mexicana* | 17 | 88% | Hepburn et al, 1994 |
| Bolivia | *L. braziliensis* | 34 | 70% | Solomon et al, 2013 |
| Bolivia | *L. braziliensis* | 18 | 89% | Soto et al, 2008 |
| Brazil | *L. braziliensis* | 61 | 51% | Romero et al, 2001 |
| Brazil | *L. guyanensis* | 57 | 26% | Romero et al, 2001 |
| Brazil | *L. braziliensis* | 30 | 53% (35.5 - 71.2) | Machado et al, 2010 |
| Brazil | *L. braziliensis, L. guyanensis, L. amazonensis* | 41 | 73% | De Paula et al, 2003 |
| Colombia | *L. guyanensis / L. panamensis* | 68 | 42% | Vega et al, 2009 |
| Colombia | *L. guyanensis / L. panamensis* | 58 | 69% | Rubiano et al, 2012 |
| Colombia | *L. panamensis / L. braziliensis* | 143 | 72% (64.3 - 79.7) | Vélez et al, 2010 |
| Colombia | *L. panamensis / L. braziliensis* | 31 | 84% (73 - 97) | Soto et al, 1998 |
| Colombia | *L. panamensis / L. braziliensis* | 23 | 91% | Soto et al, 1993 |
| Colombia | *L. panamensis / L. braziliensis* | 56 | 93% (83 - 98) | Vélez et al, 1997 |

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| Colombia | *L. panamensis* | 90 | 95% | Lopez et al, 2010 |
| Ecuador | *L. braziliensis, L. guyanensis, L. amazonensis, etc.* | 28 | 96% | Guderian et al, 1991 |
| Guatemala | *L. mexicana* | 7 | 57% | Navin et al, 1992 |
| Guatemala | *L. braziliensis* | 25 | 96% | Navin et al, 1992 |
| Guatemala | *L. braziliensis* | 22 | 90% (77 - 100) | Arana et al, 1994 |
| Panama | *L. panamensis* | 19 | 68% | Saenz et al, 1990 |
| Panama | *L. panamensis* | 19 | 100% | Ballou et al, 1987 |
| Peru | *L. braziliensis / L. peruviana* | 20 | 75% | Miranda et al, 2005 |
| Peru | *L. braziliensis* | 40 | 78% | Andersen et al, 2005 |

*When not listed, Confidence Interval (CI) not available in article.

** List of all references is provided separately.

**Table 2: Efficacy of Miltefosine in the New World in CL patients (2.5mg/Kg/day / 28 days orally)**

| Country | *Leishmania Specie* | N | Cure rate (95% CI)* | Reference** |
|---|---|---|---|---|
| Colombia | *L. panamensis* | 37 | 94% | Soto J. et al, 2001 |
| Colombia | *L. panamensis* | 49 | 91% | Soto J. et al, 2004 |
| Guatemala | *L. braziliensis* | 40 | 53% | Soto J. et al, 2004 |
| Brazil | *L. braziliensis* | 50 | 75% (64 - 86) | Machado P. et al, 2010 |
| Brazil | *L. guyanensis* | 56 | 71% (57.8 - 82.7) | Chrusciak-Tahari A. et al, 2011 |
| LA traveler's | *L. braziliensis* | 8 | 63% | Harms G. et al, 2011 |
| Colombia | *L. guyanensis* *L. panamensis* | 58 | 83% | Rubiano L. et al, 2012 |
| Colombia | *L. panamensis* *L. braziliensis* | 145 | 68.5% | Lopez L. et al, 2013 |
| Colombia | *L. panamensis* | 160 | 86.7% | Castro MD. et al 2017 |

*When not listed, CI not available in article.

** List of all references is provided separately.

Local heat, especially the one produced by radio frequency waves (ThermoMed™) has been widely tested for CL in both Old and New World. This treatment consists of applying heat of 50°C locally for a period of 30 seconds. Heat is applied to cover the whole lesion. It usually requires the use of local anesthesia. The procedure and the machine that produces heat are approved by the FDA for the treatment of CL. The advantages of using thermotherapy include: 1) high security profile; 2) requires only one application, which ensures compliance with treatment; 3) easy to use in the field, since the machine operates on batteries; 4) its effectiveness does not depend on the species of *Leishmania* causing the lesion as it is a physical measure; 5) cost, sole investment is at the beginning for the purchase of the machine, which costs approximately US$ 5,500, and 6) can be used in patients in whom systemic treatment with antimonials are contraindicated, including pregnant and breastfeeding women. In the New World, ThermoMed™ device has been evaluated in Mexico, Guatemala, Colombia and Brazil (all but one was randomized clinical trials), reporting cure rates that range from 53% to 90% (Mean 70.7%; SD 13.7%) (See table 3).

Drug combinations are commonly used for the treatment of different infectious

diseases, including malaria, tuberculosis, leprosy, and more recently, visceral leishmaniasis (VL). Paromomycin + meglumine antimoniate is currently the first line treatment for VL in Africa, while the combination of paromomycin + miltefosine is recommended as second-line treatment for VL in the Indian sub-continent. Combinations are also recommended for the treatment of CL, i.e. local application of liquid nitrogen (cryotherapy) + intralesional meglumine antimonate is one of the options recommended by WHO for the treatment of CL in countries in the Eastern Mediterranean region. In the New World, different combinations have been evaluated, i.e. systemic meglumine antimoniate + allopurinol in Colombia and Peru; imiquimod cream in combination with systemic meglumine antimoniate in Peru; paromomycin cream + systemic meglumine antimoniate in Colombia; systemic meglumine antimoniate + gamma interferon in Guatemala and systemic meglumine antimoniate in combination with topical or systemic pentoxifylline or combination of vaccines with meglumine antimoniate in Brazil.

Results from these studies are variable and range from no difference between mono and combination therapy to better efficacy with the combination. A common problem in many of these studies, however, is that combinations included compounds or approaches for which there was no strong evidence of their effectiveness when administered alone[12, 13, 14, 15,16].

**Table 3: Efficacy of Thermotherapy in the New World in CL patients**

| Country | *Leishmania Specie* | N | Cure rate (95% CI)* | Reference** |
|---|---|---|---|---|
| Guatemala | *L. braziliensis* | 14 | 64% | Navin T. et al, 1990 |
| Mexico | *L. mexicana* | 191 | 90% | Velasco et al, 1989 |
| Brazil | *L. braziliensis* | 16 | 75% | Lobo IM. et al, 2006 |
| Colombia | *L. panamensis* | 24 | 58% (49 - 66) | Lopez L. et al, 2012 |
| Colombia | *L. braziliensis* | 59 | 53% (49 - 66) | Lopez L. et al, 2012 |
| Brazil | *L. braziliensis* | 13 | 86.7% (54.63 - 97.23) | Gonçalves S., 2018 |

*When not listed, CI not available in article.

** List of all references is provided separately.

DNDi evaluated in a phase II trial the safety and efficacy of combining thermotherapy (TT) (one application, 50°C for 30 seconds) with miltefosine at the standard dose of 2.5 mg/kg/day for 21 days orally for the treatment of uncomplicated CL in Peru and Colombia. Preliminary results in the ITT analysis, after completing the enrolment of 130 patients have shown an efficacy at D90 (Initial cure) of 82% and 62% for the Combination and the TT alone arms, respectively (p= 0.02). Final results in the ITT analysis show a cure rate at D90 of 80.3% (53/66) and 57.8% (37/64) for the combination and TT alone, respectively (p = 0.0055)[17].

Originally the present study was planned to assess the non-inferiority of the combination therapy in comparison to the current recommended first line treatments, meglumine antimoniate or miltefosine monotherapy for 28 days. However, based on the revised treatment guidelines published by WHO-PAHO in 2022 in which the use of systemic antimonial received only a conditional recommendation, principally because of its well-known toxicity, and is recommended to be used only in case where there is no other option, the study protocol amendment 7 was proposed to prematurely discontinue the inclusion of additional patients in the meglumine antimoniate arm.

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| | The advantages offered by this combination are: a) using two approaches that are currently recommended for individual use, and for which there is good information regarding their efficacy and safety when used alone; b) the use of a local plus a systemic treatment would hypothetically have an additive effect, since systemic treatment would eliminate those circulating or remaining parasites located in the periphery of the lesion that local treatment fails to remove and which might be the cause of relapses[18,19]; c) it offers the opportunity to increase the current cure rate reported with any other treatment approach available when used alone; d) it will reduce the length of treatment with miltefosine and hopefully the cost and rates of adverse events associated with 28 days of treatment with miltefosine. |
| **Trial Objectives** | This randomized, open label, multi-centre, non-inferiority study aims to compare that the combination of thermotherapy (one application, 50°C for 30") and 3 weeks of miltefosine (2.5 mg/kg/day for 21 days orally) (here after referred to as combination), is non-inferior to the current recommended first line treatment, miltefosine monotherapy (2.5 mg/kg/day for 28 days orally), for uncomplicated CL cases in the NW.<br>**Primary Objective**<br>• To determine the non-inferior efficacy of the combination in comparison to the standard first line treatment miltefosine monotherapy as measured by the percentage of patients with initial clinical cure at D90.<br><br>**Secondary objectives:**<br><br>• Assess the proportion of patients who show clinical improvement at D90 (have more or equal of 75% and less than 100% re-epithelization) and achieve 100% re-epithelization at D105 (late responders).<br>• Assess the proportion of relapses at D180.<br>• Describe the proportion of patients randomized in the meglumine antimoniate arm until its discontinuation who show initial cure at D90, clinical improvement at D90 and 100% re-epithelization at D105 and relapse at D180.<br>• Assess the safety and tolerability profile for each regimen (percentage of treatment discontinuation, frequency and severity, causality with each study drug and seriousness of Adverse Events (AEs)).<br>• Assess the time to achieve 100% re-epithelialization/ flattening of ulcerated/ non ulcerated lesions by *Leishmania* species. |

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| **Trial Endpoints** | **Primary endpoint**<br>• The proportion of initial clinical cure in each regimen (Combination of TT + miltefosine, miltefosine monotherapy) measured at D90.<br>• Initial Cure: Ulcerated lesions: 100% re-epithelialization* of the ulcer(s) by D90. Non-Ulcerated lesions: flattening and/or no signs of induration of the lesion(s) by D90.<br>* Assessment of the lesion size is routinely used in all CL studies as a way to measure efficacy. The methods and ways to measure CL lesions are well known by the researchers. An SOP for this purpose has been developed and study staff will be trained to assure procedures are standardized beforehand.<br>The percentage of re-epithelialization of the ulcer(s) is calculated by comparing the size of the ulcer at D90 against D1. Measures must be taken after cleaning the lesion and removing the crust. Measures must be done in two perpendicular directions using an electronic caliper. The area of ulceration will be calculated using the area calculation for an ellipse as follows:<br>Area = A/2*B/2*π $mm^2$, where A = longest diameter of ulceration in mm; B = perpendicular to "A" diameter of ulceration in mm and π = 3.14.<br>**Secondary endpoints**<br>• The number of patients who fulfil the criteria for clinical improvement at D90 and late responders at D105.<br>• The number of patients who fulfil the criteria of initial cure at D90 or late responders at D105 and have no relapse by D180 (final cure).<br>• The number of patients randomized in the meglumine antimoniate arm until its discontinuation who fulfill the criteria of initial cure at D90, late responders at D105 and final cure at D180.<br>• Percentage of treatment discontinuation, frequency, severity, causality with each study drug and seriousness of AEs by treatment group.<br>• Proportion of lesions with 100% re-epithelialization/flattening at each measurement time point by *Leishmania sp*.<br>Initial, late responders (if required) and final cure assessments will be done in a blinded manner by the site clinicians at D90, D105 and D180, respectively. |

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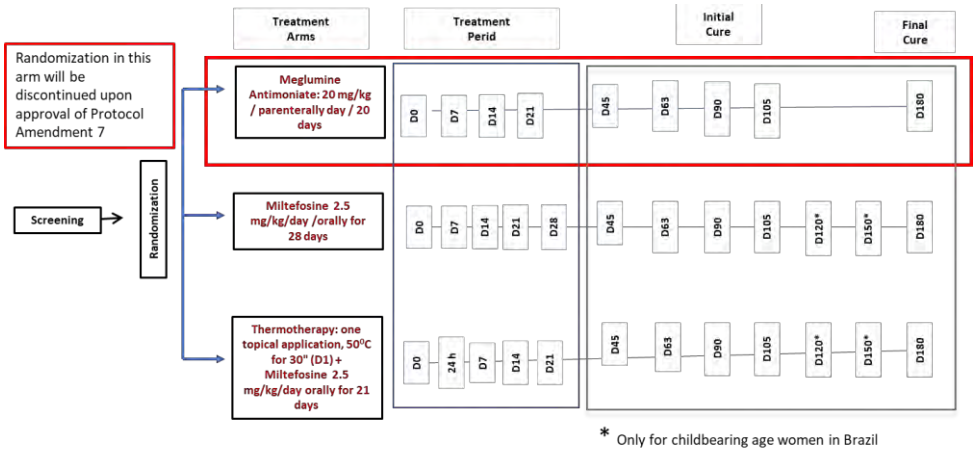
| **Trial Design** | This will be a randomized, open label, multicenter non-inferiority clinical trial in patients with uncomplicated CL from Brazil, Peru, Bolivia and Panama.<br><br>Initially there were three arms in the study:<br>- Meglumine Antimoniate, 20 mg/kg/day for 20 days parenterally,<br>- Miltefosine monotherapy 2.5 mg/kg/day for 28 days orally, and<br>- Thermotherapy (one session, 50ºC for 30" applications*) + miltefosine 2.5 mg/kg/day for 21 days orally.<br>*Depending on the size of the lesion, more than one application may be administered.<br><br>The protocol amendment 7 proposed to discontinue prematurely the meglumine antimoniate arm. Once amendment 7 of the protocol goes into effect, all new patients will be randomly allocated to receive only:<br>Investigational arm:<br>- Thermotherapy (one session, 50ºC for 30" applications*) + miltefosine 2.5 mg/kg/day for 21 days orally.<br>*Depending on the size of the lesion, more than one application may be administered.<br>Control arm:<br>- Miltefosine monotherapy 2.5 mg/kg/day for 28 days orally.<br><br>However, all study participants assigned to the meglumine antimoniate arm before protocol amendment 7 becomes effective will continue in the study and will be treated and followed up until D180.<br><br>Patients assigned to the combination treatment will start treatment at Day 1 and have a follow-up visit of 24 hours to assess safety of thermotherapy. Hereafter, these patients are required to return at Days 7, 14, 21, 45, 63, 90, 105 (late responders only) and 180 after the beginning of treatment to assess safety and efficacy. In Brazil, women of childbearing potential are required to also return on D120 and D150 to perform blood pregnancy tests. Women with irregular menstrual cycle, should return for blood pregnancy tests every two weeks until D150.<br>Patients assigned to the meglumine antimoniate treatment before discontinuation of this arm becomes effective are required to come at Days 1, 7, 14, 21, 45, 63, 90, 105 (late responders only) and 180 after the beginning of treatment to assess safety and efficacy.<br>Patients assigned to the miltefosine monotherapy are required to come at Days 1, 7, 14, 21, 28, 45, 63, 90, 105 (late responders only) and 180 after the beginning of treatment to assess safety and efficacy. In Brazil, women of childbearing potential are required to also return on D120 and D150 to perform blood pregnancy tests. Women with irregular menstrual cycle, should return for blood pregnancy tests every two weeks until D150.<br><br>Patients who have 100% re-epithelization at D90 are declared cured and appointed to come to their D180 assessment. If at D90 re-epithelization of the ulcer(s) is more or equal to 75% but less than 100%, patients will be defined as having clinical improvement and will be asked to return to D105 for a late responder assessment. |

## Diagram of study design



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| **Main Entry Criteria**<br>Inclusion | **Inclusion Criteria**<br>Patients must meet all the following criteria to be included in the study:<br>• Males and females, aged ≥12 and ≤60 years old (upper age limit according to local regulations), and weighing ≥ 30Kg.<br>• Patient with a confirmed diagnosis of CL in at least one lesion by at least one of the following methods: 1) microscopic identification of amastigotes in stained lesion tissue, or 2) demonstration of *Leishmania* by Polymerase Chain Reaction (PCR), or 3) positive culture for promastigotes.<br>• Patient has a lesion that satisfies the following criteria:<br>– Lesion size ≥ 0.5 cm and ≤ 4 cm (longest diameter).<br>– not located on the ear, face, close to mucosal membranes, or on a location that in the opinion of the Principal Investigator (PI) is difficult to apply the TT.<br>– Patient with ≤4 CL lesions.<br>– Duration of lesion less than 4 months by patient history.<br>• Patient able to give written informed consent/ assent form.<br>• In the opinion of the investigator, the patient is capable of understanding and complying with the protocol. |
| Exclusion | **Exclusion criteria**<br>Patients meeting any of the following criteria will be excluded from the study:<br>• Female with a positive urine or blood pregnancy test at screening or who is breast-feeding or female at fertile age who does not agree to take appropriate effective contraception during treatment period and up to D180 visit. In Brazil: female at fertile age who does not agree to use two effective methods of contraception: one barrier method and one highly effective method (defined in section 8.2.4) 30 days prior to the treatment onset and up to D180 visit. In Peru: female at fertile age who does not agree to use two effective methods of contraception: one barrier method and one highly effective method during treatment period up to D180 visit.<br>• History of clinically significant medical problems / treatment that might interact, either negatively or positively, with treatment of cutaneous leishmaniasis including any immunocompromising condition. Examples of significant medical problems are patients with clinical or laboratory evidence of hepatic, or renal diseases. Patients with immunocompromising conditions such as having a positive diagnosis for HIV, transplanted patients, those in treatment for auto immune diseases, patients receiving immunosuppressant, immunobiological or antineoplastic treatments.<br>• Within 8 weeks (56 days) of Day 1, received treatment for the entry lesion leishmaniasis with any medication including antimonials likely, in the opinion of the PI, to modify the course of the *Leishmania* infection.<br>• Has diagnosis or suspected diagnosis of mucocutaneous leishmaniasis based on physical exam.<br>• Has laboratory values at screening as follows:<br>o Serum creatinine: above upper normal level*.<br>o Alanine aminotransferase (ALT) or Aspartate aminotransferase (AST): 3 times above upper normal level*.<br>*Normal ranges obtained from local laboratory.<br>• Patient who is not willing to attend the study visits or is not able to comply with follow-up visits up to 6 months.<br>• Known history of addiction/ alcohol abuse. |

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| | • Hypersensitivity to miltefosine or any study medication excipients.<br>• Patients with Sjogren-Larson Syndrome. |
| **Study Duration** | The recruitment period per study site will be 13 months and the follow-up period will be 6 months (from the beginning of treatment). Therefore, from FPFV to LPLV per study site will be 19 months.<br>In Brazil, considering the time for contraception locally required for women of childbearing potential, from FPFV to LPLV per study site the entire study duration might be up to 21 months. |
| **Test Drugs** | Meglumine antimoniate solution (Glucantime®) contains 8.1% Sb5+ (81 mg/mL). The injection can be administered either intramuscularly or intravenously by infusion (over 5–10 min). It will be given at dose of 20 mg/kg, daily for 20 days with maximum daily dose of 15 mL. Its administration will be done following local treatment recommendations. This trial arm will be discontinued. However, patients assigned to this arm before protocol amendment 7 becomes effective will continue in the study and will receive complete treatment as initially planned.<br><br>Miltefosine will be given orally, together with food at a dose of 2.5 mg/kg twice a day (bid) or three times a day (tid), daily, up to maximum total dose of 150 mg/day for either 21 days (combination arm) or 28 days (monotherapy arm).<br><br>Thermotherapy: One single session at 50°C for 30” applications to all lesions. Depending on the size of the lesion, more than one application may be administered. Heat is produced with the Localized Current Field radio-frequency generating device manufactured by ThermoMed™. This unit operates with batteries that are rechargeable on household. The device produces a frequency of 6.78 mHz and has a dial on the main housing that allows for temperature selection. |
| **Statistics** | **Sample size**<br>The thermotherapy + miltefosine phase II study conducted in Colombia and Peru has shown an initial cure rate of 82% for the combination arm. The mean cure rate of meglumine antimoniate in the NW is around 75% and 70.2% for miltefosine.<br>Initially, sample size was calculated for the two one-sided test (TOST) in order to guarantee 80% power to detect a true non-inferiority of at least one of two comparisons (Combination vs Glucantime® and Combination vs Miltefosine). In this case, we took into account the worst-case scenario, which would be to compare the Combination therapy (82% efficacy) with Glucantime® (75% efficacy) using a 10% margin. Since 2 comparisons were planned in the same study, a Bonferroni correction was also applied to guarantee an overall 5% type 1 error. Using these assumptions, the original sample size was 92 patients per arm, which would be increased by 10% due to possible losses, for a final sample size of 102 per arm, 306 in total.<br><br>In protocol amendment 7, the analysis will remain as planned but comparison will be performed only between combination arm and miltefosine monotherapy arm. Since only one comparison will be performed, there is no multiplicity of testing issue and therefore, no need for adjustment of type 1 error by applying a Bonferroni correction.<br>Using assumptions of 70.2% efficacy for miltefosine monotherapy and 80.3% for combination, according to phase II final study results[17], a non-inferiority margin (10%), a power of 80%, and an alpha of 0.05 (two-sided, no adjustment for multiplicity) the required sample size is 57 patients per arm in each of the two |

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| | remaining arms. With an increase of 10% for potential losses to follow-up, final sample size is 63 per arm, 126 in total.<br><br>The total number of patients included in the trial will be approximately 180, taking into account the number of patients randomized to the meglumine antimoniate arm until discontinuation of this arm (between 45 to 55 patients).<br>A computer-generated randomization code will be used for patient treatment allocation to one of the three arms indicated and utilizing a 1:1:1 allocation ratio.<br><br>Once meglumine antimoniate arm is discontinued, randomization code in this arm will be skipped and patient will be randomized again in the online system until he/she is allocated in one of the two remaining arms, using the same list generated for the original design.<br><br>**Summary of analysis**<br>Efficacy endpoints will be presented as summary statistics by treatment group and will be conducted once all patients complete their D90/ D105 and D180 follow-up visits. The number of patients who met the criteria for cure divided by the total number of patients in that group will be presented by study group. Any patient who does not have lesion assessments at D90/D105 and D180 time points will be considered a failure in the Intent-to-treat (ITT) analysis.<br><br>Median time to the development of any Adverse Event (AE) will be determined using life-table analysis. All data will be presented separately by treatment group, local and systemic AEs. AEs 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.<br>Descriptive statistics will be used to present trial 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.<br><br>A Statistical analysis plan will be developed in advance. Parameters for definition of efficacy include the proportion of patients with initial cure, late responders and final cure in each regimen (TT + miltefosine, meglumine antimoniate and miltefosine monotherapy). Since the goal is to test for non-inferiority of the new treatment, a two one-sided test (TOST)[16,17,18] procedure will be employed. Briefly, the test consists in constructing a $(1-2\alpha)\ x\ 100\%$ confidence interval (CI) for the efficacy difference (new – current). Non-inferiority will then be established at the $\alpha$ level if the lower limit of the CI is above – δ (the margin, in this case 0.10).<br><br>No interim analysis will be performed in this trial.<br><br>Patients' data randomized in meglumine antimoniate arm until recruitment in this arm is discontinued, will be summarized in a descriptive analysis, but not included in the comparative efficacy analysis. |
| **DSMB** | A Data Safety Monitoring Board (DSMB) meeting is scheduled to take place at the start of the study, at an ad-hoc basis during the study and at the end of the study, as per defined on the DSMB Charter. The identity of patients included in these analyses will not be provided to DSMB experts to preserve confidentiality. |

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