TREATMENT OF MUCOCUTANEOUS LEISHMANIASIS WITH MILTEFOSINE

Protocol No. PLB-MILT-201

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1 LIST OF ABBREVIATIONS AND DEFINITIONS

AE	adverse event
ALT	alanine transaminase
BUN	blood urea nitrogen
CL	cutaneous leishmaniasis
CRF	case report form
CTC	Common Terminology Criteria
DCL	diffuse cutaneous leishmaniasis
DNA	deoxyribonucleic acid
ENT	ear-nose-throat
ECG	electrocardiogram
FDA	food and drug administration
IND	investigational drug application
IRB	institutional review board
mITT	Modified Intention-to-treat
ML	mucosal leishmaniasis
MSS	mucosal severity score
PCR	polymerase chain reaction
PE	physical examination
PI	Principal Investigator
SAE	serious adverse event

2 STUDY SCHEDULE

Time and Event Schedule									
Study Phase	**Screening[a]**	**Treatment**				**Follow-up**			
Study Day/Week/Month	**Day -14 to -1**	**Day 1**	**Day 7 ± 3 days**	**Day 14± 3 days**	**Day 28**	**Week 6**	**Month 3**	**Month 7**	**Month 13[h]**
Informed Consent	X								
Demographics	X								
Medical History[b, c]	X	X	X	X	X	X	X	X	X
Physical examination[d]	X	X			X	X	X	X	X
Leishmanial History and Exam[c]	X				X	X	X	X	X
Hematology	X								
Blood Chemistries	Xe			X[f]	X[f]	X[g]	X[g]	X[g]	X[g]
ECG	X								
Pregnancy Test	X	X	X	X	X		X	X	
Eligibility Checklist	X								
AEs		X	X	X	X	X	X	X	X
Treatment		Daily							

[a]Screening includes the 14-day period for conducting screening procedures prior to the first administration of investigation product on Day 1.
[b]Includes medication history
[c]If subject does not appear at the scheduled Week 6, Month 3, Month 7, or Month 13 visits, these data may be obtained from the subject by telephone, and will be noted as obtained in that manner.
[d]Includes vital signs
[e]Screening laboratories
[f]Alanine transaminase (ALT) and creatinine on Day 28 and also on nominal day 14
[g]If abnormal on previous examination
[h]ML subjects only

3 ABSTRACT

The purpose of this Treatment Protocol is to make miltefosine immediately available for mucocutaneous leishmaniasis subjects presenting in the United States. Submission of an application for New Drug Approval is in process. Enrollment in this protocol makes it possible for subjects and their physicians to utilize miltefosine prior to approval.

If entrance criteria are met, subjects with mucosal or cutaneous leishmaniasis will receive miltefosine at a targeted dose of 2.5 mg/kg/day for 28 days. During the 28-day treatment period, the subject will return to the treatment facility at least three times for evaluations and recording of any AEs. Blood samples for the evaluation of transaminase and creatinine levels will be drawn at the midpoint and at the end of therapy.

Subjects will return to the treatment facility to be examined clinically at Study Week 6 (i.e., 2 weeks after the end of therapy), Month 3 (2 months after therapy), and Month 7 (6 months after treatment) for mucosal leishmaniasis (ML) and cutaneous leishmaniasis (CL) subjects. ML subjects will also be evaluated at Month 13 (12 months after treatment).

OBJECTIVES

Primary: Record the efficacy of miltefosine, approximately 2.5 mg/kg/day for 28 days, in the treatment of mucocutaneous leishmaniasis presenting in the United States.

Secondary: Record the tolerance of miltefosine, approximately 2.5 mg/kg/day for 28 days, in the treatment of mucocutaneous leishmaniasis presenting in the United States.

STUDY DESIGN

Open-label, single group, multicenter study.

POPULATION

1. Male or female at least 18 years of age.
2. Weight at least 30 kg.
3. Provided informed consent
4. ML or CL already demonstrated in at least one lesion by at least one of the following methods:
    a. positive culture of lesion material for promastigotes,
    b. microscopic identification of amastigotes in stained lesion tissue, or
    c. PCR of lesion material.
5. No antileishmanial therapy in the previous 4 weeks.
6. Subject capable of understanding and complying with the protocol.
7. If female and of child-bearing potential, a negative pregnancy test during screening and agree to use an acceptable method of birth control during the treatment phase and for 6 months after treatment.
8. If female, not breast feeding.
9. No clinically significant medical disorder. Specifically:
    a. Platelet count >100 x 10^9/L
    b. Leukocyte count >3 x 10^9/L

c. Hemoglobin >10 g/dL
d. ALT <2 times upper limit of normal range
e. Bilirubin <1.5 times upper limit of normal range
f. Serum creatinine <1.5 times upper limit of normal range
g. Major surgery within last 2 weeks
h. Any non-compensated or uncontrolled condition

10. No known Sjogren-Larssen syndrome

INVESTIGATIONAL PRODUCT

Drug name: Miltefosine (50 mg capsules).
Dosing regimen: Approximately 2.5 mg/kg/day (maximum: 150 mg per day) for 28 days.
Dose: The daily dosage for subjects of body weight 30-45 kg is 100 mg miltefosine (2 capsules Impavido 50 mg). Subjects with a body weight higher than 45 kg receive 150 mg miltefosine daily (3 capsules Impavido 50 mg). The dose is administered orally, with meals.

STUDY PROCEDURES

Subjects with ML or CL from which *Leishmania* have already been identified are potentially eligible to be treated with miltefosine via this protocol. Treating Physicians with potentially eligible subjects will contact the protocol Principal Investigator (PI), and receive the case report forms (CRF) from the PI. The Treating Physician will complete the screening CRF pages for demographics, medical history, leishmaniasis history, clinical laboratory results that are available, and identification of *Leishmania* in the lesion, and send the completed CRF pages to the PI. If after PI review, the subject is potentially eligible for the protocol, the PI will send the protocol, the miltefosine package insert, the informed consent form, and a blank copy of FDA form 1572 to the Treating Physician. Although this protocol will have already been approved by a "central" Institutional Review Board (IRB), if there is an additional need to have the Treating Physician's local IRB approve the protocol, the Treating Physician will obtain the approval, and obtain informed consent from the subject. The rest of the laboratory tests must be accomplished so that all screening laboratory tests are completed prior to enrolling a potential subject. If in the physician's opinion the subject appears eligible for enrollment, the Treating Physician will send to the PI the local IRB signature page (if needed), protocol signature page, informed consent signed by both the subject and the Treating Physician, the rest of the completed CRF pages for screening, and the form 1572 completed with the Treating Physician's information plus the Treating Physician's curriculum vitae. After the PI's review of these forms, the investigational product will be sent from the drug repository to the Treating Physician for that subject's use.

Treatment will be daily for 28 consecutive days. During treatment at weeks 1, 2, and 4, the subject will return to the treatment facility to be assessed for adverse events and to receive additional supply of medication if needed. Compliance with drug administration will be assessed by subject interview and pill count. Blood for transaminase and creatinine values will be drawn at the midpoint and at the end of therapy.

Subjects will return to the treatment facility to be examined clinically at Study Week 6, Study Months 3 and 7 months for ML and CL subjects, and also at Study Month 13 for ML subjects.

SAMPLE SIZE AND STUDY DURATION:
10-20 subjects per year for 1-5 years.

OUTCOME PARAMETERS:
Efficacy: Clinical response of lesions
Safety: AEs

ANALYSIS:
Efficacy: The percent of subjects who have clinical cure of all lesions will be calculated.
Safety: The occurrence and severity of AEs will be described.

4 INTRODUCTION AND RATIONALE

4.1 Mucocutaneous Leishmaniasis (Murray et al., 2005)

4.1.1 Clinical Spectrum

Sandflies inoculate the skin with flagellated *Leishmania* promastigotes, which invade or are phagocytosed by local and immediately recruited host cells. Within phagolysosomes of resident macrophages, surviving promastigotes transform and replicate as amastigotes, which infect additional macrophages either locally or in distant tissues after dissemination.

Multiple *Leishmania* species produce CL, primarily *L. major, L. tropica,* and *L. (L.) aethiopica* (Old world cutaneous leishmaniasis); *L. infantum* and *L. chagasi* (Mediterranean and Caspian sea regions); and *L. mexicana, L. (L.) amazonenesis, L. braziliensis, L. (V.) panamensis, L. (V.) peruviana,* and *L. (V.) guyanensis* (New World CL).

A papule typically begins at the sandfly bite, enlarges to a nodule, and ulcerates over 1–3 months. Flat plaques or hyperkeratotic or wart-like lesions also develop in Old World disease. Subjects, including travelers and military personnel, first seek attention because of one to two, or sometimes several (up to dozens) non-healing skin lesions on nocturnally exposed skin. In Old World CL, most lesions are papules, nodules, or nodule-ulcers, whereas ulcerative lesions are most common in New World CL. Disseminated lesions and localised lymphadenopathy preceding skin ulcers occur in Brazil. *L. tropica* infection disseminates in that new papules can appear around a healed lesion (leishmaniasis recidivans).

Mucosal dissemination of New World species (*L. braziliensis, L. panamensis, L. guyanensis*) occurs in 1–10% of infections, developing 1–5 years after cutaneous leishmaniasis has healed, but sometimes coincident with active skin lesions; about 90% of subjects have a preceding cutaneous scar. ML typically begins with erythema and ulceration at the nares, proceeding to nasal septum perforation and destructive inflammatory lesions. The latter can obstruct the pharynx or larynx and produce remarkable disfigurement. Mucosal disease is occasionally reported outside of Latin America, and can be acquired by travelers.

4.1.2 Diagnosis

Diagnosis is routinely made microscopically by identification of amastigotes in biopsies, scrapings, or impression smears. Combination of microscopy and culture increases diagnostic sensitivity to more than 85%, and culture (or DNA analysis) allows species identification. Detection of parasite DNA in lesion material by polymerase chain reaction (PCR), although generally only performed in research settings, is usually most sensitive in the diagnosis of both cutaneous and mucosal leishmaniasis.

4.1.3 Treatment with Classical Agents

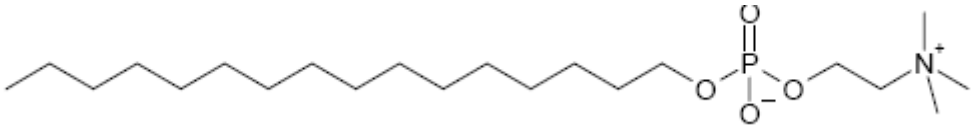
Cutaneous disease heals by re-epithelialization with scarring. Most Old World lesions self-cure within 2–4 months (*L. major*) or 6–15 months (*L. tropica*). In New World CL, self-healing after 3 months is rapid in *L. mexicana* (~75%), but slow in *L. braziliensis* (about 10%) and *L. panamensis* infections (about 35%).

CL is treated to accelerate cure, reduce scarring especially at cosmetic sites, and to attempt to prevent dissemination (eg, mucosal disease) or relapse. Treatment is especially likely to be given for persistent lesions (>6 months) or lesions that are located over joints, multiple (5–10 or more), or large (4–5 cm or more). Classic treatment with parenteral antimony would probably be successful in CL in all regions; however, up to 20 daily injections of this moderately toxic drug approximates the morbidity of self-healing disease itself.

ML can produce potentially life-threatening inflammatory disease and must be treated. The standard regimen, 28 days of parenteral antimony, induces cure in around 75% of cases of mild disease (nares only), but advanced disease responds less well. Amphotericin B can be used as rescue therapy.

4.2 Miltefosine

Miltefosine (hexadecylphosphocholine) is a phosphatidylcholine analogue. The structural formula of miltefosine is:



The drug was registered in India in 2002 and in Germany in 2004 to treat visceral leishmaniasis. Miltefosine was registered in Columbia in 2005 to treat visceral and cutaneous leishmaniasis, and post 2005 to treat CL and ML in Bolivia and in many other countries in the Americas. As the only recognized oral agent for leishmaniasis, the years since 2005 have been spent in further studies of visceral leishmaniasis and other forms of leishmaniasis.

4.2.1 Dosage and Administration

Miltefosine is provided as capsules for oral administration, containing 50 mg of drug substance per capsule.

List of excipients: Collodial anhydrous silica, microcrystalline cellulose, lactose monohydrate, talc, magnesium stearate, gelatin, titanium dioxide, ferric oxide, purified water.

Recommended dosage: Approximately 2.5 mg/kg/day orally for 28 days. The daily dosage for subjects of body weight 30-45 kg is 100 mg miltefosine (2 capsules Impavido 50 mg). Subjects with a body weight greater than 45 kg receive 150 mg miltefosine daily (3 capsules Impavido 50 mg).

The capsules should be taken with meals. Subjects taking 2 capsules per day should take one capsule with the morning meal and with the evening meal. Subjects taking 3 capsules per day should take one capsule with the morning meal, the noon meal, and the evening meal.

4.2.2 Contraindications

- Hypersensitivity to the active substance or any of the excipients
- Pre-existing severe damage of liver or kidney function

- Sjögren-Larsson-Syndrome
- Pregnancy and women of childbearing potential who do not use reliable contraception during and up to 3-6 months after treatment

4.2.3 Warnings and Precautions

Impavido is contraindicated in pregnancy. Women of childbearing potential have to use effective contraception during and up to 3-6 months after treatment. Vomiting/diarrhea are very common/common side effects of therapy with Impavido and can compromise the efficacy of oral contraception. The subject must be informed by her physician of these symptoms and if necessary, suitable alternative methods of contraception must be used.

Subjects with ALT (liver function), serum creatinine, and blood urea nitrogen (BUN) (renal function) 1.25 times above the normal range were generally excluded from the clinical studies. Thus, data from subjects with abnormalities of liver and kidney function are not available.

Treatment with Impavido may lead to an increase in serum creatinine. Kidney function must be evaluated in biweekly intervals. In subjects with clinically significant abnormality in kidney function, monitoring should be continued until normalization.

Gastrointestinal symptoms such as nausea, vomiting, diarrhea, and a feeling of motion sickness are possible side effects of therapy with Impavido. The subjects must be instructed that in case of prolonged persistence of vomiting and diarrhea a sufficient fluid intake must be ensured, to avoid dehydration and consequently the risk of an impaired renal function.

4.2.4 Adverse Reactions

Organ system	**Very Common** **>10% of subjects**	**Common** **1-10% of pts**
Gastrointestinal	Nausea Vomiting Grade 1-2*	Motion sickness Diarrhea Grade 1* Headache
Renal	Creatinine elevation Grade 1*	

*Grade = National Cancer Institute Common Terminology Criteria (CTC) grade

4.2.5 Drug Interactions

In vitro investigations have shown that interactions are unlikely with medications that are metabolized by cytochrome P450 or glucuronised or otherwise conjugated. However, the possibility of interactions with commonly used medicinal products cannot entirely be excluded.

4.2.6 Use in Specific Populations

Pregnancy: There are not adequate data on the use of miltefosine in pregnant women. Studies in animals have shown reproductive toxicity. Impavido is contraindicated in pregnancy. Women of childbearing potential have to use effective contraception during and up to 3-6 months after treatment. Vomiting/diarrhea are very common/common side effects of therapy with Impavido and can compromise the efficacy of oral contraception. The subject must be informed by her

physician of these symptoms and if necessary, suitable alternative methods of contraception must be used. The subject has to be advised to immediately contact her physician for pregnancy testing as soon as there is any suspicion of pregnancy. If the test is positive, the physician and subject must discuss the risks associated with this pregnancy.

Nursing mothers: It is not known whether miltefosine is excreted in the milk. Impavido must not be used during lactation; otherwise breast feeding must be stopped.

Geriatric use: Few subjects of age 65 yrs or greater have been included in investigations.

Renal impairment: Subjects with kidney function tests (serum creatinine, BUN) 1.25 times above the normal range were generally excluded from the clinical studies. Thus, data from subjects with mild/moderate/severe abnormalities of kidney function are not available.

Hepatic impairment: Subjects with liver function tests (AST) 1.25 times above the normal range were generally excluded from the clinical studies. Thus, data from subjects with mild/moderate/severe abnormalities of hepatic function are not available.

4.2.7 Clinical and Non-clinical Pharmacology

4.2.7.1 Pharmacodynamic Properties

Miltefosine has a marked direct antileishmanial activity *in vitro* and in animal models. However, the relevance of specific values (ED50, ED90) to clinical efficacy is unknown. The specific mode of action of miltefosine in leishmaniasis is unknown. Among other possible mechanisms, miltefosine can inhibit the metabolism of phospholipids in cell membranes of parasites.

Nonclinical Pharmacokinetic Studies

Distribution studies in rats, using radioactively labeled miltefosine, showed highest uptake of radioactivity in kidney, liver and spleen. Slow elimination of radioactivity from tissues (half lives 8-16 days) is partially explained by metabolism of miltefosine and incorporation of the labeled choline fragment into physiological lipids.

No oxidative metabolism by 15 different cytochrome P450 isozymes was observed *in vitro*. No CYP3A induction by miltefosine was found *in vivo*, in rats. Thus, no interaction has to be expected between miltefosine and drugs, like contraceptive hormones, that are metabolized by CYP3A. A slow metabolic breakdown could be shown in human hepatocytes, resulting in the release of choline by phospholipase D like cleavage of the miltefosine molecule. The fatty alcohol containing fragment of miltefosine can enter the metabolism of fatty acids after being oxidized to palmitic acid. This oxidation is blocked in subjects with Sjögren-Larrson syndrome, which is caused by a genetic defect in fatty aldehyde dehydrogenase activity. Preclinical and clinical studies suggest that only a very minor part of the administered dose will be excreted as the unchanged drug substance. Instead, choline and choline-containing metabolites are the most likely excretion products.

Pharmacokinetic studies in subjects with cutaneous leishmaniasis (Dorlo et al., 2008)
Subjects were administered miltefosine at an average dosage of 1.8 mg/kg/day. Pharmacokinetic analysis in this CL subject population resulted in calculation of two half lives: a first half-life of 7.0 days and a terminal half-life of 31 days.

Nonclinical Toxicology
Toxicological studies with miltefosine have been performed in mice, rats, dogs and rabbits. Adverse reactions not observed in clinical studies, but seen in animals at exposure levels similar to clinical exposure levels and with possible relevance to clinical use are described in the section below.

Acute and Chronic Toxicity
The oral administration of miltefosine in rats was associated with regressive and/or progressive lesions especially affecting the eyes (retinal degeneration), kidneys (acute resp. chronic nephropathy) and organs with rapidly dividing cell tissues (atrophy/hyperplasia), as well as reproductive organs (atrophy). These alterations were observed after 8 weeks treatment at doses of 10 mg/kg/day which led to plasma drug levels of about 52 µg/ml. Juvenile rats were more sensitive than adult rats to the miltefosine induced effects, especially on eyes and kidneys.

Reproductive Toxicity
Testicular atrophy and impaired fertility were observed in rats following daily oral doses of 8.25 mg/kg. These findings were reversible within a recovery period of 10 weeks. Reproductive toxicity studies in rats during the early embryonic development (up to day 7 of pregnancy) indicate an embryotoxic, fetotoxic and teratogenic risk following miltefosine dosages of 1.2 mg/kg/day and higher. Embryo- and fetotoxic findings were also observed in rabbits after oral administration of miltefosine during the phase of organogenesis (2.4 mg/kg/day and higher).

Mutagenicity/Carcinogenicity
Miltefosine tested negative in 6 of 7 of mutagenicity tests (AMES-Salmonella test, DNA-amplification test, chromosomal aberration test *in vitro*, UDS-test *in vivo*/*in vitro*, oral mouse-micronucleus test *in vivo*). The V 79 mammalian cell HPRT gene mutation test showed an increase in mutant frequency without dose dependency. In view of all mutagenicity test results, the single positive finding in the V 79 HPRT test is considered to be not of toxicological relevance with respect to a mutagenic risk to humans. The results of the mutagenicity tests ruled out a genotoxicity-mediated carcinogenic potential of miltefosine. Carcinogenicity studies were not performed.

4.2.8 Clinical Studies in Mucocutaneous Leishmaniasis

In a dose-ranging study against *L. panamensis* in Colombia, high rates of cure were seen in subjects treated with 2.5 mg/kg/day dose for 3-4 weeks, the same regimen previously used for visceral leishmaniasis. The regimen of 2.5 mg/kg/day x 28 days was then evaluated in a placebo-controlled study in both Colombia against *L. panamensis* and Guatemala against *L. braziliensis/L. mexicana*. Success in this placebo-controlled study led to continent-wide and world-wide use of miltefosine for all forms of leishmaniasis.

Further studies in the New World have been reported for Bolivian CL and Bolivian ML due to *L. braziliensis* and against Venezuelan diffuse cutaneous leishmaniasis due to *L. amazonensis*.

4.2.8.1 Dose-finding Study in *L. panamensis* Region in Colombia (Soto *et al.*, 2001)

The cure rate for evaluable subjects in group 1 (50 mg miltefosine/day) was 9/14 (64%). The cure rate in group 2 (100 mg miltefosine/day) was 12/18 (67%). The cure rate in subjects given 150 mg miltefosine/day [2.5 mg/kg/day for these subjects of average weight of 67 kg] was 14/14 (100%) for subjects treated for 20 days in group 3 and 16/18 (89%) for subjects treated for 28 days in group 4. Because a larger total dose of drug should be more effective in the general population, the dose of approximately 2.5 mg/kg/day for 28 days was adopted based on this study.

4.2.8.2 Placebo Controlled Study in *L. panamensis* Region in Colombia and in *L. braziliensis/L. mexicana* Region in Guatemala (Soto *et al*., 2004)

The objective of this study was to demonstrate that miltefosine, 2.5 mg/kg/day for 28 days, was superior to placebo in cutaneous leishmaniasis in male or female subjects >12 yrs when assessed 6 months after end of treatment. The per protocol cure rate against *L. panamensis* in Colombia was 91% (**Table 1** below), in conformity to the results from groups 3 and 4 of the dose-ranging study #3092, and statistically higher than the cure rate for placebo (38%).

Table 1. Efficacy of Miltefosine Used to Treat CL in Colombian and Guatemalan Subjects

	Colombian site		Guatemalan site	
Variable	**Miltefosine recipients (n = 49)**	**Placebo recipients (n = 24)**	**Miltefosine recipients (n = 40)**	**Placebo recipients (n = 20)**
No. of subjects cured	40	9	20	4
No. of subjects with treatment failure				
All	4	15	18	15
Parasite-positive lesions	0	15	12	12
Size of lesion doubled	2	5[a]	4[b]	2
Relapse	2	0	4	1
No. of unassessable subjects				
All	5	0	2	1
Lost after therapy	2	0	1	1
Lost after 2 weeks	2	0	1	0
Lost after 3 months	1	0	0	0
Cure rate, n/N (%)				
Intent-to-treat	40/49 (82)	9/24 (38)	20/40 (50)	4/20 (20)
Per-protocol	40/44 (91)	9/24 (38)	20/38 (53)	4/19 (21)

[a] All 5 of these subjects also had parasite-positive lesions.
[b] Two of these 4 subjects also had parasite-positive lesions.
Table adapted from Soto *et al*., 2004.

The cure rate was comparable to that historically achieved for pentavalent antimony in Colombia. Although the per protocol cure rate in Guatemala (53%) was statistically higher than placebo cure rate (21%), the cure rate against *L. braziliensis* in this locale was less than that historically achieved for pentavalent antimony in Guatemala (>90%).

Because this study was placebo-controlled, the rate of subjective and laboratory AEs specifically due to miltefosine can be determined. The percent of subjects with noteworthy subjective AEs, and with abnormal laboratory findings, is shown in **Table 2** below:

Table 2. Subject Demographics and Efficacy results (Soto et al., 2008)

	No. (%) of subjects	
Variable	**Miltefosine recipients (n = 89)**	**Placebo (n = 44)**
Treatment-emergent AEs		
Nausea	32 (36)	4 (9)[a]
Motion sickness	26 (29)	10 (23)
Headache	24 (27)	9 (20)
Vomiting		
≥ 1	28 (31)	2 (5)[a]
1-2	22 (25)	1 (2)
3-4	3 (3)	1 (2)
>4	3 (3)	0 (0)
Diarrhea		
≥1	5 (6)	1 (2)
1-2	4 (4)	1 (2)
>2	1 (1)	0 (0)
Laboratory parameters		
Creatinine level		
Increased	29 (33)	4 (9)[b]
CTC grade 1[c]	28 (31)	4 (9)
CTC grade 2[d]	1 (1)	0 (0)
Elevated alanine aminotransferase level	7 (8)	8 (18)
Elevated alanine aminotransferase level	9 (10)	5 (11)

[a] $p<0.001$
[b] $p=0.003$
[c] less than 1.5 times the upper limit of normal
[d] less than 1.5 and 3.0 times the upper limit of normal

This placebo-controlled study of 89 miltefosine subjects shows that in humans who are without systemic disease, nausea and vomiting but not diarrhea occur significantly more frequently than in placebo subjects, and elevation of serum creatinine but not ALT occurs significantly more frequently than in placebo subjects. Most of the miltefosine-specific AEs were of mild severity: 80% of the episodes of vomiting were CTC grade 1; 97% of the creatinine elevations were CTC grade 1.

4.2.8.3 Comparison of Miltefosine to Pentavalent Antimony in *L. braziliensis* Region in Bolivia (Soto et al., 2008)

The subjects were randomized to treatment with miltefosine (2.5 mg/kg/d for 28 days) or to a standard course of intramuscular pentavalent antimony (Glucantime: 20 mg/kg/d for 20 days) in 2:1 allocation.

AEs were as expected for the two drugs. The primary AE for the miltefosine group was gastrointestinal symptoms, which were experienced by 27 of 44 subjects (61%) for a median of 3 days (range, 1–10 days). For the glucantime group, 13 of 18 subjects (72%) reported arthralgias and/or local pain at the injection site for a median of 7 days (range, 5–14 days). Efficacy data is presented in the **Table 3** below.

Table 3. Subject Demographics and Efficacy Results (Soto *et al.*, 2008)

	Miltefosine recipients	**Glucantine subjects**
Presenting characteristics		
Number of subjects	44	18
Age of subjects [years; median (range)]	27 (12-57)	23 (12-51)
Male/Female	36/8	15/3
Weight of subjects [kg; median (range)]	59 (39-78)	59 (38-80)
Number of ulcers	64	30
Number per subject [median (range)]	1 (1-3)	1 (1-3)
Area (mm^2) [median (range)]	181 (10-3,172)	150 (4-900)
Results		
End treatment		
No. of subjects healed/failed/lost	15/0/0	9/0/1
Percent of subjects healed/evaluable	15/44=34%	9/17=53%
1 month after treatment		
No. of subjects healed/failed/lost	31/0/0	16/0/2
Percent of subjects healed/evaluable	31/44=70%	16/16=100%
3 months after treatment		
No. of subjects healed/failed/lost	39/4/1	15/1/2
Percent of subjects healed/evaluable	39/43=91%	15/16=94%
6 months after treatment		
No. of subjects healed/failed/lost	36/5/3	15/1/2
Percent of subjects healed/evaluable	36/41=88%	15/16=94%

*P=0.01 (Fisher exact test).
† P=0.67 (Fisher exact test).

The final cure rate for miltefosine (88%) was insignificantly less than the value for pentavalent antimony (94%), although antimony was more rapidly curative since the cure rate at 1 month after therapy was higher for antimony (100%) than for miltefosine (70%). This comparator-controlled study vs. *L. braziliensis* in Bolivia shows that the relative lack of efficacy of miltefosine for *L. braziliensis* in Guatemala may be an outlier.

4.2.8.4 Single-arm Study on Diffuse Cutaneous Leishmaniasis (DCL) in Venezuela (Zerpa et al., 2007)

DCL is essentially untreatable because of an inappropriate immunological response by the host to parasite antigens. Sixteen subjects with DCL refractory to multiple previous treatments were treated with miltefosine, 2.0–2.5 mg/kg daily, for long periods of time (75–218 days).

AEs were very mild. Side-effects were observed in four subjects; two complained of nausea and vomiting and reduced the dose without consultation, with improvement in symptoms. One subject presented with dizziness on the second day of treatment. On day 20 one subject presented with urticaria, that improved with systemic antihistamines. None presented with alteration of laboratory parameters during the study.

Subjects showed dramatic clinical improvement and reduction in the parasite burden by day 15 after the initiation of treatment, which continued while treatment was maintained. By day 45, 15 subjects showed 80–90% clinical improvement. Nevertheless, suspension of treatment was followed by the development of new lesions between 30 and 120 days later in all but one subject. This study shows that miltefosine treatment can be tolerated for months beyond the normally recommended period of 4 weeks.

4.2.8.5 Mucosal Leishmaniasis (Soto et al., 2007)

The initial study design was a randomized equivalency study of oral miltefosine (50 subjects) versus standard therapy with pentavalent antimony (25 subjects). Due to changes in regional standard of care to amphotericin B (45 mg/kg as 1mg/kg doses for 45 consecutive days), and due to initial positive response rates in the miltefosine group, subjects refused to be treated with antimony or to be randomized to the amphotericin B arm of the study. Therefore, the final study design became an evaluation of one cohort of 78 subjects who received miltefosine (2.5 mg/kg/day for 28 days). An almost contemporary group of 19 subjects who received amphotericin B (45 mg/kg) as miltefosine treatment was getting organized was evaluated as a *post hoc* comparison group.

Miltefosine was well tolerated. Nausea, vomiting, and diarrhea were each reported by 8–17 subjects. Most episodes were CTC grade 1; one episode was grade 3. Most episodes lasted 1–2 days and a few lasted 3–4 days. Mean values of liver function tests and kidney function tests did not change; a few subjects had values that were slightly above the upper limit of normal after treatment.

Of the 78 subjects who received miltefosine, 72 were evaluable. Fourteen of 19 subjects in the amphotericin B group were evaluable. Fifty-one (71%) of the 72 evaluable miltefosine subjects were “cured” by the definition of >90% diminution in the mucosal severity score with 12 months of follow up. Almost all (49 [96%]) of these subjects demonstrated complete resolution of their

clinical signs. The cure rate for the 36 subjects who had "mild" disease (i.e., affecting only the nasal skin and nasal mucosa) was 83%. The cure rate for the 36 subjects who had more extensive disease (involving the palate, pharynx, and larynx) was 58%.

The cure rate for the amphotericin B group (45 mg/kg over 90 days) was 7 (50%) of 14. Since the historic cure rate for treatment of ML in neighboring Peru varies between 10% and 75%, this study suggests that miltefosine is approximately as effective as standard treatment with antimony, and with amphotericin B, for Andean mucosal disease. Before-and-after photographs of one subject from this study with involvement of the external nares (**Figure 1**), illustrates the clinical improvement in skin and mucosa consequent to successful miltefosine therapy.

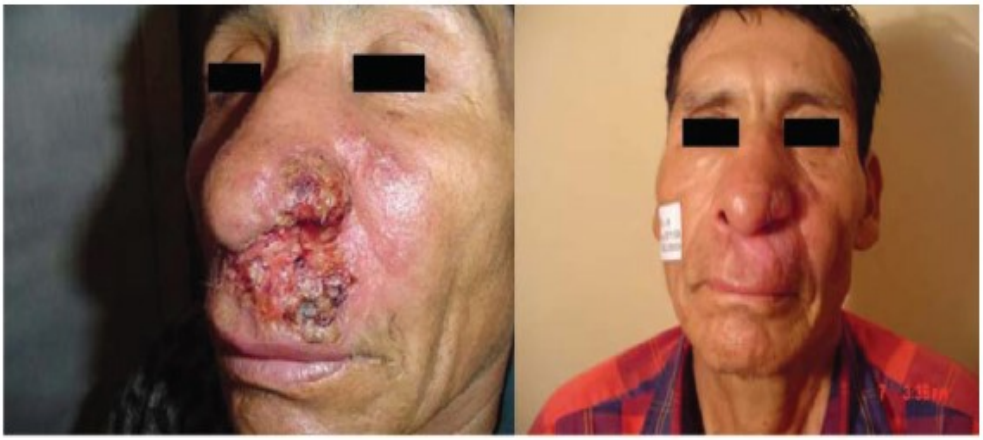


Figure 1. Patient 9 before treatment *(left)* and immediately after treatment for 28 days with miltefosine *(right)*

4.2.8.6 Clinical Summary

The cure rate for CL treated with miltefosine (2.5 mg/kg/day for 28 days) is superior to placebo and, except in one region, comparable to standard therapy with antimony. This regimen of miltefosine also gives cure rates for ML comparable to historic values for antimony, and provides remarkable clinical improvement for DCL subjects.

Subjects with CL or ML are systemically normal. For these hosts, laboratory abnormalities due to miltefosine treatment consist of the common occurrence of low grade elevations in serum creatinine, and the uncommon occurrence of higher-grade elevations, both of which are reversible. The symptoms of nausea, vomiting, and perhaps motion sickness/headache are also common.

Miltefosine has a favorable therapeutic index for CL and ML, with the additional advantage of being orally administrable. No other accepted antileishmanial agent, whether an investigational agent in the United States or registered agent elsewhere, is orally administrable.

4.2.9 References

Investigator's Brochure. Edition 15 January 2009. Paladin Labs Inc.

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5 STUDY OBJECTIVES

5.1 Primary

Record the efficacy of miltefosine, 2.5 mg/kg/day for 28 days, in the treatment of mucocutaneous leishmaniasis presenting in the United States.

5.2 Secondary

Record the tolerance of miltefosine, approximately 2.5 mg/kg/day for 28 days, in the treatment of mucocutaneous leishmaniasis presenting in the United States.

6 STUDY DESIGN

Open-label, single group, multicenter study

7 STUDY SUBJECTS

7.1.1 Estimated Number of Subjects

10-20 per year for 1-5 years

7.1.2 Inclusion Criteria

To be eligible for the study, the following must be answered "YES" or not applicable as appropriate for the study subject:

1. Is the subject a male or female at least 18 years of age?
2. Does the subject weight at least 30 kg?
3. Does the subject have a diagnosis of ML or CL in at least one lesion by at least one of the following methods:
    a. positive culture of lesion material for promastigotes,
    b. microscopic identification of amastigotes in stained lesion tissue, or
    c. PCR of lesion material?
4. In the opinion of the investigator, is the subject capable of understanding and complying with the protocol?
5. If female and of child-bearing potential, did the subject have a negative pregnancy test during screening and agree to use an acceptable method of birth control during the treatment phase and for 6 months after treatment is completed?
6. Has the subject signed informed consent?

7.1.3 Exclusion Criteria

To be eligible for the study, the following must be answered "NO" or not applicable as appropriate for the study subject:

1. Is the subject a female who is breast-feeding?
2. Does the subject have a clinically significant medical disorder?
    a. Platelet count $<100 \times 10^9$/L
    b. Leukocyte count $<3 \times 10^9$/L
    c. Hemoglobin <10 g/dL

d. ALT, >2 times upper limit of normal range
e. Bilirubin >1.5 times upper limit of normal range
f. Serum creatinine >1.5 times upper limit of normal range
g. Major surgery within last 2 weeks
h. Any non-compensated or uncontrolled condition

3. In the last 4 weeks up to the present, has the subject received other treatment for leishmaniasis, including any medication with pentavalent antimony; amphotericin B, paromomycin, or imidazoles?

8 INVESTIGATIONAL PRODUCT

8.1 Description

Miltefosine is formulated as capsules of 50 mg strength. The ingredients are as follows:

Active Principle:	50 mg miltefosine
Inactive ingredients:	Lactose D10, avicel PH 101, talkum, aerosil V200, magnesium stearate

The investigator or authorized person assigns the box to an eligible subject by writing the subject's study number and alpha code on the label on the box, i.e. capsules in a box are for use in a single subject only; residual capsules may not be used for another subject. The trial medication may not be used after the retest or expiry date which is specified in the certificate of analysis.

8.2 Labeling

Each box of miltefosine contains 56 capsules: 8 packs of blisters, with each blister pack containing 7 capsules. Each box has a preprinted label with a space for the subject's ID number, alpha code, dosing instructions, 24/7 emergency telephone number, storage conditions, precautions, and lot# and expiry date, as follows:

Clinical Study # PLB-MILT-201 Sponsor: Paladin Labs (USA) Inc.
ID # _____ Alpha code _____
Take 1 capsule ____ times a day with meals
If medical emergency, call your doctor at ( ) _____
Store in original container at room temperature
CAUTION: INVESTIGATIONAL NEW DRUG LIMITED BY FEDERAL LAW TO INVESTIGATIONAL USE.
Caution: Keep out of the reach of children
Lot # 8J7717 Expiry Date: 10 July 2012
Manufactured by: Haupt Pharma Amareg GmbH, Regensburg, Germany

Each blister pack is preprinted with “Impavido 50 mg capsules” as well as other identifying information.

8.2.1 Storage

Medication should be kept out of the reach of children and away from direct sun light, and at temperatures not above 40°C (104°F).

8.2.2 Drug Accountability

The Treating Physician has to account for all used and unused trial supplies and has to complete a Medication Accountability Form. Residual supplies will be destroyed by the Treating Physician as directed. The completed Medication Accountability Form will be returned to the Data-Management Center. The trial medication must not be used outside this protocol.

8.3 Start of Treatment

Treatment should be started within 2 weeks after the completion of screening assessments. Otherwise compliance with eligibility criteria has to be reassessed.

8.4 Administration of Investigational Product (Miltefosine)

Route: Oral administration

Dose: The daily dosage for subjects of body weight 30-45 kg is 100 mg miltefosine (2 capsules Impavido 50 mg). Subjects with a body weight higher than 45 kg receive 150 mg miltefosine daily (3 capsules Impavido 50 mg).

The capsules should be taken with meals. Subjects taking 2 capsules per day should take one capsule with the morning meal and with the evening meal. Subjects taking 3 capsules per day should take one capsule with the morning meal, the noon meal, and the evening meal.

Duration of treatment: 28 days.

8.5 Allocation of Study Treatment to Subject and Compliance

For subjects who will receive 2 capsules per day, the subject will receive a box of medication (56 capsules) at the initiation of therapy which is sufficient for the total treatment period of 28 days. For subjects who will receive 3 capsules per day, the subject will receive a box of medication (56 capsules) at the beginning of therapy that is enough for 18 days of treatment, and will return to the treatment facility after 2 weeks to receive the final 2 week's supply of medication. Compliance with drug administration will be assessed by subject interview and pill count.

8.6 Treatment Modifications and Study Discontinuation

Dose reduction due to AEs: A subject who does not tolerate the planned dosage will go off-treatment. No dose reduction is planned.

Discontinuation of study treatment and withdrawal from protocol for other reasons: A subject may withdraw from the study at any time and for any reason without penalty, if he or she wishes to do so. The Treating Physician may withdraw a subject if continuing participation is believed to be harmful to the subject's well-being or in the event of protocol violations, non-compliance, positive pregnancy test during active treatment, serious intercurrent illness, or apparent failure of efficacy. The sponsor, PI, and IRB have the right to discontinue the investigation at any time.

In the event that the subject withdraws from the study before the completion of the 28 days of treatment, s/he will be asked to come to the clinic for a final assessment including AEs, lesion measurements, vital signs, blood creatinine, and concomitant medication use.

If the subject withdraws during the follow-up period, s/he will be contacted, if possible, and requested to come to the clinic for lesion measurements.

If possible, positive pregnancy should be followed to the end of the pregnancy.

8.7 Concomitant Therapy

All concomitant medications and any therapies (prescribed and non-prescribed) must be recorded on the CRF. Any change during the study should be documented as well. Symptomatic treatment of side effects and treatment of concomitant diseases not associated with leishmaniasis is permitted. Treatment with other agents known or likely to be active against *Leishmania* (pentavalent antimony, amphotericin B, paromomycin, imidazoles, local heat or cold therapy) is not permitted.

8.8 Rescue Treatment

Anti-leishmaniasis drugs must not be administered while the subject is on protocol. If the subject is removed from the protocol for any reason (decision that the subject has failed treatment, inability of the subject to comply with the protocol conditions, or desire of the subject, physician, Sponsor, or IRB), the subject may be treated with another antileishmanial agent off-protocol at the discretion of the treating physician.

9 STUDY PROCEDURES

Time and Event Schedule									
Study Phase	**Screening[a]**	**Treatment**				**Follow-up**			
Study Day/Week/Month	**Day -14 to -1**	**Day 1**	**Day 7 ± 3 days**	**Day 14± 3 days**	**Day 28**	**Week 6**	**Month 3**	**Month 7**	**Month 13[h]**
Informed Consent	X								
Demographics	X								
Medical History[b, c]	X	X	X	X	X	X	X	X	X
Physical examination[d]	X	X			X	X	X	X	X
Leishmanial History and Exam[c]	X				X	X	X	X	X
Hematology	X								
Blood Chemistries	Xe			X[f]	X[f]	X[g]	X[g]	X[g]	X[g]
ECG	X								
Pregnancy Test	X	X	X	X	X		X	X	
Eligibility Checklist	X								
AEs		X	X	X	X	X	X	X	X
Treatment		Daily							

[a]Screening includes the 14-day period for conducting screening procedures prior to the first administration of investigation product on Day 1.
[b]Includes medication history
[c]If subject does not appear at the scheduled Week 6, Month 3, Month 7, or Month 13 visits, these data may be obtained from the subject by telephone, and will be noted as obtained in that manner.
[d]Includes vital signs
[e]Screening laboratories
[f]Alanine transaminase (ALT) and creatinine on Day 28 and also on nominal day 14
[g]If abnormal on previous examination
[h]ML subjects only

9.1 Subject Recruitment

The medical community will be alerted to the availability of miltefosine via this Treatment IND via informal communications and listing on ClinicalTrials.gov.

Subjects with mucosal or cutaneous lesions from which *Leishmania* have already been identified are potentially eligible to be treated with miltefosine via this protocol. Treating Physicians with potentially eligible subjects will contact the protocol PI, and receive the CRFs from the PI. The Treating Physician will complete the screening CRF pages for demographics, medical history, leishmaniasis history, clinical laboratory results that are available, and identification of *Leishmania* in the lesion, and send (mail or fax) these CRF pages to the PI. If after PI review, the subject is potentially eligible for the protocol, the PI will send the protocol, the miltefosine package insert, the informed consent form, and the FDA form 1572 to the Treating Physician. Although this protocol will have already been approved by a "central" IRB, if there is an additional need to have the Treating Physician's local IRB approve the protocol, the Treating Physician will obtain the approval, and obtain informed consent from the subject. The rest of the laboratory tests must be accomplished so that all screening laboratory tests are completed prior to enrolling a potential subject. If in the physician's opinion the subject appears eligible for enrollment, the Treating Physician will send the local IRB signature page (if needed), protocol signature page, informed consent signed by both the subject and the Treating Physician, the rest completed CRF pages for screening data to the PI, and the form 1572 completed with the Treating Physician's information plus his/her curriculum vitae. After the PI's review of these documents, the investigational product will be sent from the drug repository to the Treating Physician for that subject's use.

Note that because the tests needed to satisfy the entrance criteria of medical history, leishmaniasis history, and identification of *Leishmania* in the lesion are standard of care for this disease, the tests can be performed prior to signing the consent form for this protocol. This procedural order [evaluate certain entrance criteria, then achieve local IRB approval if needed and signing of consent, then evaluate the rest of the entrance criteria] is chosen so that subjects do not undergo excess procedures and expectations if the subject ultimately is unlikely to be able to enroll in the protocol.

9.2 Screening Procedures

Subjects will have the following procedures performed during a two week period prior to the initiation of investigational product:

1. Consent form completed and signed.
2. Demographics form completed.
3. Medical history form completed and record medications used in the past 2 weeks.
4. Physical examination form completed including vital signs, height, and weight.
5. Leishmaniasis history and physical form completed.
    a. Record history of leishmaniasis including;
        (1) CL: estimated date that the lesion(s) first appeared, evolution of symptoms, treatments to date
        (2) ML: estimated date that the symptoms first appeared, evolution of symptoms, treatments to date

b. CL subject examination: For each cutaneous lesion, record the size of the lesion ulceration (length/width), characteristics of the lesion (ulcerated versus non-ulcerated), and anatomical location. Examination of nasal skin, nasal mucosa, palate, and pharynx must be performed by the Treating Physician.
c. ML subject examination: Examination of mucosal membranes. Each of 5 possible sites (nasal skin, nasal mucosa, palate, pharynx, and larynx) will be evaluated for 4 possible signs of disease (erythema, edema, infiltration, and erosion) by an ear, nose, and throat (ENT) specialist.

6. Pregnancy test from completed for women of child-bearing potential.
7. Hematology form completed to include white blood cells (WBC), neutrophil count, hemoglobin, hematocrit, and platelet count.
8. Chemistry form completed including glucose, sodium, potassium, creatinine, ALT, and bilirubin in the serum.

9.3 Assessment During Miltefosine Treatment

9.3.1 Study Day 1

Study Day 1 visit is conducted on the first day of miltefosine therapy and includes:

1. Medical History - record changes in medical history or medications since screening visit
2. Physical Examination form - perform brief physical exam including vital signs
3. Pregnancy test form - perform a pregnancy test in women of child bearing potential, unless the screening pregnancy test was performed within the previous 24 hours.
4. Complete the eligibility checklist to ascertain if the subject still meets all of the eligibility criteria, then the subject will receive the first dose of medicine.
5. Record any AEs

9.3.2 Study Days 7 and 14

Two visits are scheduled to occur between Study Day 2 through Study Day 27 and should preferably occur at the end of Study Week 1 and Study Week 2 with in a three-day window either side of Day 7 and Day 14. At these two visits the following assessments are performed:

1. Medical History - The subject will return to clinic for evaluation; record any AEs
2. Treatment form – complete log
3. If more medication than that which was dispensed on day 1 is needed, further medication will be dispensed on the nominal day 14 visit
4. Chemistry – record values of ALT and creatinine in the serum (Day 14 visit only)
5. Record any AEs

9.3.3 Study Day 28

Study Day 28 visit is conducted the last day of miltefosine therapy and includes:

1. Medical History - record changes in medical history or medications
2. Physical examination – record vital signs
3. Interval History of leishmaniasis:
    a. CL - lesion reactions
    b. ML - evolution of symptoms
4. CL subjects: Re-examination of each CL lesion and re-recording the size of the lesion ulceration (length/width), other notable characteristics of the lesion

5. ML subjects: Re-examination of mucosal membranes
6. Pregnancy test in women of child-bearing potential
7. Chemistry – record values of ALT and creatinine in the serum
8. Record any AEs

9.3.4 Study Week 6

Two weeks after the Study Day 28 visit record the following:

1. Medical History - record changes in medical history or medications since 28-day visit
2. Physical examination - perform brief physical exam if appropriate, and vital signs
3. Interval History of leishmaniasis since last visit to include:
    a. ML - re-examination of mucosal membranes. Evolution of symptoms
    b. CL subjects - re-examination of each CL lesion and record the size of the lesion ulceration (length/width), other notable characteristics of the lesion
4. ALT and creatinine: Repeat if abnormal on last examination
5. Record any AEs

9.3.5 Study Month 3

Study Month 3 visit is conducted 2 months after the end of miltefosine therapy and includes:

1. Medical History - record changes in medical history or medications since 6-week visit
2. Physical Examination - perform brief physical exam if appropriate, and vital signs
3. Interval History of leishmaniasis since last visit to include:
    a. ML - re-examination of mucosal membranes. Evolution of symptoms.
    b. CL subjects - re-examination of each CL lesion and record the size of the lesion ulceration (length/width), other notable characteristics of the lesion
4. Pregnancy test in women of child bearing potential
5. ALT and creatinine: Repeat if abnormal on last examination
6. Record any AEs

9.3.6 Study Month 7

Study Month 7 visit is conducted 6 months after the end of miltefosine therapy. Final follow up visit for CL subjects and includes:

1. Medical History - record changes in medical history or medications since 3-month visit
2. Physical Examination - perform brief physical exam if appropriate, and vital signs
3. Interval History of leishmaniasis since last visit to include:
    a. ML - re-examination of mucosal membranes. Evolution of symptoms
    b. CL subjects - re-examination of each CL lesion and record the size of the lesion ulceration (length/width), other notable characteristics of the lesion
4. Pregnancy test in women of child bearing potential
5. ALT and creatinine: Repeat if abnormal on last examination
6. Record any AEs

9.3.7 Study Month 13

Study Month 13 visit is conducted 12 months after the end of miltefosine therapy. Final follow up visit for ML subjects and includes:

1. Medical History - record changes in medical history or medications since 7-month visit
2. Physical Examination - perform brief physical exam if appropriate, and vital signs

3. Interval History of leishmaniasis since last visit to include:
    a. ML - re-examination of mucosal membranes. Evolution of symptoms.
    b. CL subjects - re-examination of each CL lesion and record the size of the lesion ulceration (length/width), other notable characteristics of the lesion
4. ALT and creatinine: Repeat if abnormal on last examination
5. Record any AEs

NOTE: if the subject does not return to clinic for the post-treatment visits, medical history and interval history of leishmaniasis may be obtained by telephone. Further, the subject's examination of a CL lesion may be obtained by telephone. Telephonic data will be noted as such on the CRF.

10 EFFICACY

NOTE: Responses in subjects with CL and ML will be presented separately.

10.1 Efficacy Assessments of CL Subjects

10.1.1 Assessment Methods

10.1.1.1 Parasitological Diagnosis of Leishmaniasis

Prior to entrance into this protocol, lesion(s) will have been scraped, aspirated, or biopsied; and the specimens will have been smeared onto microscope slides for examination with DifQuik or Giemsa staining, placed in culture medium to visualize motile promastigotes, or subjected to PCR analysis for *Leishmania*-specific nucleic acid.

NOTE: The Treating Physician may choose to perform these parasite diagnostic procedures on lesions that are not-healed post therapy or on a new lesion to see if the lesion contains *Leishmania*. These procedures are at the Treating Physician's discretion and not formally part of this protocol.

10.1.1.2 Examination of Cutaneous Lesions

Ulcerated CL lesions will be measured in mm for the longest diameter and perpendicular width of ulceration. Non-ulcerated lesions will be measured for length and width of the raised area of the lesion. Lesion area = Length x Width. Lymph nodes in the drainage path of the cutaneous lesion will be noted and measured.

10.1.1.3 Examination of Mucosa

For CL subjects, the Treating Physician will examine the nasal and oral mucosa of each subject.

10.1.2 Definitions of Lesion Response

Healed: 100% reduction in lesion area [lesion size = 0x0 mm^2]
Improved: 50% - 99% reduction in lesion area
No change: 49% enlargement to 49% reduction in lesion area
Worse: >50% enlargement of lesion area at or after the end of therapy.
Relapsed: Substantial enlargement of lesion after initial improvement or healing.

10.1.3 Definition of Clinical Responses

Lesion Cure: Lesion is healed at the Month 7 visit.
Lesion Failure: Each lesion will be considered a failure if it does not meet the criteria for cure (missing data will be considered failures). The intended response to therapy is a progressive diminution in lesion size and no relapse. Lesion failure therefore is defined as clinically worse at the end of therapy or thereafter, OR does not improve at the Week 6 or Month 3 visit, OR is not healed at the Month 7 visit, OR relapses.

NOTE: Parasitological responses can assist the clinician in evaluating the clinical response of the lesion. If the Treating Physician has parasitologically re-investigated a slowly responding lesion

at time-points greater than 2 weeks after the end of therapy, and parasites are still present by microscopy or culture, the physician may interpret the data as indicating therapeutic failure. Note that the presence of parasites prior to 2 weeks after the end of therapy should not be interpreted. Note that because PCR is more sensitive than microscopy or culture on which the clinical experience in the literature is based, PCR positivity post therapy cannot easily be interpreted.

10.1.4 Definition of Subject Cure

Subject Cure: All lesions cured by the end of follow up at Month 7 visit.
Subject Failure: Not all lesions clinically cured, or a new lesion due to *Leishmania*.

Primary endpoint(s): The co-primary endpoints are the subject cure rate for the modified intention-to-treat (mITT) and evaluable populations.

10.2 Efficacy Assessment of ML Subjects

10.2.1 Assessment Methods

For subjects with suspected ML, an ENT specialist will examine the nasal and oral mucosa. Each of 5 possible sites (nasal skin, nasal mucosa, palate, pharynx, and larynx) will be evaluated for 4 possible signs of disease (erythema, edema, infiltration, and erosion) and graded on a 0-3 scale: 0 = no disease, 1 = mild disease, 2 = moderate disease, 3 = severe disease. If possible, the ENT specialist who first examined the subject will re-evaluate the oral-nasal mucosa at each follow-up visit for the subject

10.2.2 Clinical Parameter and Definition of Clinical Responses

Clinical response will be measured will be a composite score, the mucosal severity score (MSS). The MSS is the sum of the severity scores for each clinical sign at each clinical site of disease. The Maximum MSS is 60: grade 3 severity for each of 4 possible signs at each of the 5 possible sites.

Healed: MSS that is 0 in absolute value [equals 0% of the entrance MSS score].
Improved: MSS that is 1%-25% of the entrance MSS score.
Non-substantial Change: MSS that is 26%-99% of the entrance MSS score.
Worse: MSS >100% of the entrance MSS score.
Relapse: MSS substantially increases after initially improving or becoming 0.

10.2.3 Definition of Subject Responses

Subject cure: Clinical cure is lesion is healed.
Subject failure: Leasion did not heal. NOTE: The intended response to therapy is a progressive diminution in signs and symptoms and no relapse. Subject failure is defined as clinically worse at the Week 6 visit or thereafter, OR does not improve at the Month 3 or month 7 visits, OR is not healed at the Month 13 visit, OR relapses.

Primary endpoint(s): The primary endpoints are the clinical cure rates for the mITT and evaluable population.

10.3 Withdrawal from Protocol for Lack of Efficacy

The Treating Physician may choose to withdraw the subject from the protocol for lack of efficacy and to initiate rescue (alternative) therapy for ML or CL.

For CL, the basis for lack of efficacy will generally be failure of at least one lesion: lesion has worsened at the end of therapy or thereafter, OR does not Improve at the Week 6 or Month 3 visit, OR is not healed at the Month 7 visit, OR relapses, or a new lesion.

For ML, the basis for lack of efficacy will generally be failure: clinically worse (by MSS score) at the Week 6 visit of or thereafter, OR does not improve at the Month 3 or Month 6 visit, OR is not healed at the Month 13 visit, OR relapses.

11 SAFETY/ADVERSE EVENTS

11.1 Assessment Methods

11.1.1 Symptoms

Medical history: At screening, during treatment, and during follow up, subjects will be asked general questions about their well being and specific questions about gastrointestinal distress (nausea, vomiting, diarrhea). If a medically substantial problem is reported, it should be reported to the Treating Physician immediately.

11.1.2 Signs

At screening, a physical exam of the head, eyes, ears, cardiovascular system, lungs, abdomen (liver/spleen), extremities, skin, lymphatic system, neuropsychiatric mental status and sensory/motor status, musculoskeletal system and general appearance will be performed. Height and weight will be recorded. Vital signs (blood pressure, heart rate, temperature) will be recorded. During treatment and follow up, a targeted physical exam will be performed if prompted by the subject or the subject's appearance. Vital signs will be recorded.

11.1.3 Laboratory Assessments

Venous blood will be collected during screening for measurement of hematology parameters to be reported [white blood cells (WBC), neutrophil count, hemoglobin, hematocrit, and platelet count] and chemistry parameters including glucose, sodium, potassium, creatinine, ALT, and bilirubin. These baseline values will be used to determine if the subject has a concomitant medical condition.

On nominal days 14 and 28, a blood sample will be collected for AST and creatinine only. On follow up visits at Month 3, Month 7, and Month 13, blood will again be collected for ALT and creatinine if on the previous sample the ALT value was above the upper limit of normal, or if the creatinine clearance was abnormal. Serum creatinine levels will be used to calculate creatinine clearance (CrCl) according to the Cockroft-Gault formula as follows:

Males $$\text{CrCl (mL/min)} = \frac{[140 - \text{age (years)} \times \text{body weight (kg)}]}{(72) \times (\text{serum creatinine [mg/dL]})}$$

Females $$\text{CrCl (mL/min)} = \frac{0.85 \times [140 - \text{age (years)} \times \text{body weight (kg)}]}{(72) \times (\text{serum creatinine [mg/dL]})}$$

11.1.4 Pregnancy Test

For females of child-bearing potential, an FDA-cleared serum or urine pregnancy test that measures human β-chorionic gonadotropin will be used during screening, at the end of treatment, and during follow up.

11.1.5 Electrocardiogram (ECG)

An ECG will be taken during screening to evaluate if the subject has a baseline medical condition.

11.2 AE Definition

An AE is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial, whether or not the event is considered investigational product-related or clinically significant. For this study, AEs will include events reported by the subject, as well as clinically significant abnormal findings on physical examination or laboratory evaluation.

A new illness, symptom, sign, or clinically significant clinical laboratory abnormality is considered an AE. A worsening of a pre-existing condition or abnormality is also considered an AE. The appearance of scars at healed lesion sites will not be reported as an AE. Stable chronic conditions which are present prior to clinical trial entry and do not worsen are not considered AEs.

11.3 Severity Definitions

Subjective AEs will be graded according to the definitions provided below:

Grade 1: Mild symptoms invoking a minimum degree of discomfort that are easily tolerated.

Grade 2: Moderate symptoms that result in a reduction in normal daily activity, but is not totally incapacitating. This may or may not require medical intervention.

Grade 3: Severe symptoms that may be totally incapacitating or result in marked reduction in normal daily activity. Medical intervention is usually required.

Grade 4: Potentially life-threatening event that requires emergency intervention or hospitalization.

Physical examination AEs and laboratory AEs will be graded according to the National Cancer Institute's CTC for AEs. For the expected AEs of vomiting, diarrhea, ALT elevation, and creatinine elevation, the Common Terminology Criteria are:

Analyte	Grade 1	Grade 2	Grade 3	Grade 4
ALT elevated	>ULN – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 20.0 x ULN	>20.0 x ULN
Creatinine	>ULN - 1.5 x ULN	>1.5 - 3.0 x ULN	>3.0 - 6.0 x ULN	>6.0 x ULN
Vomiting	1 episode per day	2 to 5 episodes per day	6 to 10 episodes per day	more than 10 episodes per day
Diarrhea	increase of 2 to 3 stools per day	increase of 4 to 6 stools per day	7 to 9 stools per day	10 or more stools per day

The Common Terminology Criteria for unanticipated AEs will be accessed if needed.

11.4 Assessment of Causality

The relatedness of an AE to the investigational product is the best estimate of the causal relationship between the investigational product and an AE at the time of reporting. Causality will be assessed by the Treating Physician and subsequently by the PI.

Definitions are:

Unrelated: There is no temporal relationship between the event and the administration of the investigational product or the event is clearly due to the subject's medical condition, other therapies, or accident.

Unlikely: There is evidence of exposure to the investigational product but there is another more likely cause of the event.

Possibly Related: There is some temporal relationship between the event and the administration of the investigational product and the event is unlikely to be explained by the subject's medical condition or other therapies.

Probably Related: The temporal relationship between the event and the administration of the investigational product is compelling, and the subject's medical condition and other therapies cannot explain the event.

Definitely Related: The event follows a reasonable temporal sequence from administration of the medication or follows a known or suspected response pattern to the medication.

The categories of Definitely Related, Probably Related, and Possibly Related will be considered investigational product related with regards to summary statistics.

11.5 AE Actions and Outcomes

For each AE that is reported, the actions taken with respect to investigational product can be:

1. none
2. permanently discontinued

Also, outcomes will be recorded as:

1. resolved
2. resolved with sequelae
3. ongoing
4. required treatment
5. unknown

11.6 Monitoring and Reporting of Adverse Events

The Treating Physician will monitor subjects for the occurrence of AEs from time the first investigational product is taken on Day 1 through the end of follow up at Month 7 for CL or Month 13 for ML. For the period between Study Day 1 and Study Week 6 (2 weeks after the end of therapy), all AEs regardless of seriousness or relationship to the investigational product are to

be recorded on the CRF. For the period Week 6 to Month 7 for CL, or Month 13 for ML, only AEs requiring medical attention are to be recorded on the CRF.

Whenever possible, symptoms should be grouped as a single syndrome or diagnosis. The Treating Physician should specify the date of onset, maximum severity grade, his/her opinion as to association between the AE and the administration of the investigational product (causality), outcomes including corrective therapy given (if applicable). Clinically significant AEs ongoing at the last follow up visit will be followed to resolution or stabilized including the administration of any concomitant medications.

11.7 Serious Adverse Event (SAE)

Each AE or reaction will be classified by the Treating Physician as serious or non-serious. Based on the seriousness of the AE or reaction appropriate reporting procedures will be followed.
An SAE is defined as:

- results in death;
- is life-threatening; (NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.)
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity; or
- is a congenital anomaly/birth defect.

In addition, important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug reaction, when based on appropriate medical judgment that may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in the above definition. For this protocol, becoming pregnant between Study Day 1 and Month 7 is considered to be an SAE.

11.8 SAE Reporting Requirements

The Treating Physician must report all SAEs to the PI that are possibly, probably, or definitely related to study medication within 24-hours of learning of the SAE by email, telephone, or fax.

Within a further 3 working days, the SAE CRF, along with any other appropriate supporting documentation, must be submitted by the Treating Physician to the PI.

The Data Management Center will prepare a narrative or a MedWatch (FDA Form 3500A) if the SAE meets the criteria for expedited reporting for an SAE.

12 ANALYTICAL PLAN

12.1 Sample Size

As the study is not designed to compare the efficacy of the two treatments, there is no formal sample size calculation.

12.2 Analytical Populations

mITT Population: The mITT population includes all subjects who received any administration of an investigational product.

Evaluable Population: The evaluable population will include all subjects who received daily doses of investigational product for at least 25 of the total of 28 days, had lesion measurements at the Month 7 for CL or the month 13 visit for ML, and who did not receive rescue medications to treat leishmaniasis at any time during the study.

Efficacy outcomes will be presented separately for subjects with CL and ML.

Safety Population: The safety population includes all subjects who received any administration of investigational product.

12.3 General Analytical Procedures

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.

12.4 Analysis of Baseline Data

By-subject and summaries of the baseline data will be provided for the mITT, and evaluable populations.

12.5 Analysis of Treatment Compliance Data

By subject and summaries of compliance with scheduled treatments will be provided, including total exposure (number of days and total dose) to investigational product.

12.6 Analysis of Efficacy Outcomes of CL Subjects

12.6.1 Lesion Area Measurements

Summary statistics for each subject will include lesion area and change from baseline at each measurement time point.

12.6.2 Lesion and Subject Cure Rate

For each subject, the proportion of lesions at each measurement time point that have healed, improved, did not change, worsened, or relapsed will be calculated. Also for each subject, the proportion of all lesions that cured and all lesions that failed will be presented. The proportion of subjects who cure or fail will be calculated. This data will be presented for the mITT and evaluable populations.

12.6.3 Handling of Missing Data

Missing lesion measurements will be ignored when presented as summary statistics of lesion areas and lesion responses as presented by numbers of lesions evaluated at each time point. Subjects who do not provide lesion response measurements at their final scheduled follow up will be considered lesion ane subject failures in the mITT analysis.

Note that if a subject does not appear at the scheduled Study Week 6, Month 3, or Month 7 visits, the subject's estimate of his/her lesion response may be obtained from the subject by telephone, and will be noted as obtained in that manner. Such data is not defined as missing. For such data, the Treating Physician and the PI will jointly decide if the subject's estimate is likely to be reliable. In a single-group non-comparative study such as this one, errors in interpretation will only apply to miltefosine subjects and will not be corrected by being equally applied to a comparator group. Therefore, it is important for subject, physician, and Investigator to avoid two potential errors: 1) erroneously viewing a lesion as healed when in fact it is still present, 2) erroneously viewing a lesion as failed because a subject does not show up for clinic, when in fact the lesion has healed and the subject does not want to spend time coming to clinic.

12.7 Analysis of Efficacy Outcomes of ML Subjects

12.7.1 Lesion assessments

Summary statistics for each subject will include the individual components of the MSS score and the total MSS score, and change from baseline in both parameters, at each measurement time point.

12.7.2 Subject Cure Rate

The proportion of subjects at each measurement time point with clinical cure, improvement, fail, and relapse will be calculated. Data will be presented for the mITT and evaluable populations.

12.7.3 Handling of Missing Data

Missing lesion measurements will be ignored when presented as summary statistics of lesion areas and lesion responses as presented by numbers of lesions evaluated at each time point. Subjects who do not provide lesion measurements at their final scheduled follow up will be considered clinical failures in the mITT analysis.

Note that because the subject cannot accurately examine his/her oro-nasal membranes, subject reports of physical mucosal examination cannot be accepted.

12.8 Analysis of Safety Data

Symptoms (subjective AEs) will be coded using the most recent version of the Medical Dictionary of Regulatory Affairs (MedDRA) by assigning a preferred term and will be grouped by system, organ, and class (SOC) designation. Listings of each individual AE including start date, stop date, severity, relationship, duration, and outcome will be provided.

Laboratory data and physical signs (including vital signs) will be presented as summary statistics by Study Day including changes from baseline as well as in by-subject data listings. A summary and listing of analyte concentrations and vital signs for subjects with levels outside normal laboratory limits will also be presented. Clinically significant AEs, SAEs, discontinuation due to AEs, and other significant AEs will be individually listed and narrated.

The above safety analysis will be provided for the mITT and Evaluable populations.

13 ADMINISTRATIVE AND REGULATORY STANDARDS

An IND will be obtained from the FDA. The PI will sign a Form FDA 1572.

13.1 Ethical Review of Protocol

The PI will obtain protocol approval from a central IRB before starting the study.

13.2 Informed Consent

Each subject's Treating Physician will be responsible for obtaining informed consent from that subject prior to his/her participation. If that subject's treatment will occur in a facility over which an IRB has responsibility and that IRB requires review of the protocol, the Treating Physician will obtain approval from that IRB prior to the subject's participation in the protocol.

13.3 Responsibility for Subject Care

The Treating Physician is responsible for the care of his/her subject.

13.4 Treatment Cost

Miltefosine will be provided by the Sponsor at no cost; however, subject care is the responsibility of the Treating Physician and no payment for subject care can be made by the Sponsor.

13.5 Protocol Exemption Committee

The PI and the Sponsor's Representative will constitute the protocol exemption committee.

14 STUDY DOCUMENTATION/DATA MANAGEMENT

The data management plan and a statistical analysis plan will be prepared by the data management center.

14.1 Subject Identification (ID) Code

Each subject will be assigned a subject ID code which will be provided with the screening CRF package. The subject ID code will consist of a three digit numeric code and a three digit alphabetical (alpha) code. The first digit of the numeric code will be a sequential number starting with 101. The alpha code will be a random sequence of three letters. The Subject ID code will be used on all CRFs, and laboratory data sheets.

14.2 Data Collection and Monitoring

Data will be collected at the study site in the subject's medical records, which will be transcribed at the site on to the study CRFs provided by the data management center. CRFs are to be completed on an ongoing basis according to the instructions in the Study Procedures Manual.

The Treating Physician will be responsible for completing the CRFs, verifying the accuracy of the CRF data versus the source documentation, and sending the completed CRFs to the data management center.

The Treating Physician will correspond with the PI at the following time points:

- prior to obtaining informed consent to verify subject eligibility,
- Study Day 28 or Week 6 follow-up visit,
- Month 7 follow up for CL subjects, or Month 7 and 13 for ML subjects.

Completed CRFs should be submitted to the data management center prior to the Treating Physician contacting the PI by telephone. The purpose of these communications between Treating Physician and PI is to assure optimum subject care and conveyance of study data.

The Treating Physician and PI can also correspond with each other at any other time of their choosing.

In spite of these interactions and advice that may be offered by the PI, clinical responsibility for subject care rests with the Treating Physician.

14.3 Data Editing and Control

Data received at Fast-Track will be reviewed prior to being entered into the main study database. If incomplete or inaccurate data are found, a data clarification request will be forwarded to the clinical site for a response. The site will resolve data inconsistencies and errors prior to returning data to Fast-Track. Errors must be corrected by drawing a single line through the incorrect entry and by writing the new value as close to the original as possible. The correction must then be initialed and dated. All corrections and changes to the data will be reviewed prior to being entered into the main study database

14.4 Data Analysis

A final analysis of all clinical trial data will be performed when all of the data for every subject has been collected, entered into the main study database, audited, and locked for analysis. A final clinical study report will be prepared in accordance with International Committee on Harmonization E3 Structure and Content of Final Clinical Study Reports.

14.5 Publication

The results of the full study may be published at the discretion of the sponsor. The Treating Physician may publish or present the data of his/her patients provided that the publication/presentation does not disclose any of the Sponsor's Proprietary Information. The Treating Physician agrees to submit the draft of any proposed publication/presentation to Sponsor at least thirty (30) days prior to submission for publication/ presentation, and agrees, at the request of Sponsor**,** to withhold any such submission for an additional period, not to exceed ninety (90) days, to allow Sponsor to file patent applications or to take any other action designed to protect its patent rights. No personal data will be used in any external communication or publication.

14.6 Subject Confidentiality

To maintain subject confidentiality, all records and CRFs specifically generated by this protocol will be identified using the subject ID code. Protocol generated records will be stored in a secure location and only the protocol staff will have access to the records.

15 ROLES AND RESPONSIBILITIES OF STUDY PERSONNEL

15.1 Principal Investigator

Signs the protocol agreement, below, and the Form FDA 1572. The PI is responsible for passage of the protocol and any amendments through a "central" IRB before starting the study. The PI is responsible for interaction with Treatment Physicians to ensure protocol adherence and data integrity, and overall coordination of the study.

The PI is also responsible for participating in safety evaluations of subjects during the conduct of the study. The PI will review all unanticipated problems involving risk to subjects or to others, SAEs, and all subjects' deaths associated with the protocol, and provide an unbiased written report of the event. At a minimum, the PI should comment on the outcomes of the event or problem and, in the case of a SAE or death, comment on the relationship to participation in the study. The PI should also indicate whether he/she concurs with the details of the report provided by the Treating Physician. Reports for SAEs determined by either the Treating Physician or PI to be possibly, probably, or definitely related to participation and reports of events resulting in death should be promptly forwarded to the Sponsor and the "central" IRB.

The PI is responsible for filing a final clinical study report with the Sponsor.

15.2 Associate Investigator

Associate investigators are responsible for performing the PI's duties in his/her absence. In all sections of this protocol, "PI" is taken to mean "PI or Associate PI in the absence of the PI".

15.3 Treating Physicians

This protocol involves the clinical use of an investigational agent. Treating Physicians are responsible for implementation of protocol with respect to his/her subject: briefing potential participants, obtaining proper informed consent, obtaining IRB approval if needed, determining study eligibility based on inclusion and exclusion criteria, accountability and proper storage of the investigational product, administering the product to the patients, reporting of any AEs or protocol deviations, entering and verifying CRF data, reporting study data to the Data Management Center via CRFs, and corresponding with the PI at scheduled time periods.

The Treating Physician is considered an "investigator" in the regulatory sense, and has to supply a Form FDA 1572 and curriculum vitae to the PI so that these documents can be sent to the FDA.

15.4 Data Management Center

Prepare and provide CRFs to clinical site; design, develop, and validate the clinical trial database; perform data entry into the clinical trial database; perform database quality control and data analysis.

16 STATEMENT OF AGREEMENT

The PI has carefully read this protocol and agrees to oversee the clinical study as described in a professional and competent manner in accordance with the generally accepted standards of Good Clinical Practice.

The Treating Physician has carefully read this protocol and agrees to oversee the clinical treatment as described in a professional and competent manner in accordance with the policies of the medical institution where the Treating Physician conducts the clinical treatment and any applicable local, state, and agency laws and regulations. The Treating Physician understands that the IND has been submitted the protocol to FDA. The Treating Physician agrees to cooperate with and be available to regulatory authorities as needed regarding the conduct of this clinical study.

The Treating Physician further attests that he/she has obtained informed consent from the subject to be treated under this protocol.

17 SIGNATURES

_____ _____

Principal Investigator Date
Jonathan Berman, M.D., Ph.D.

_____ _____

Sponsor Date
Robert K. Vinson, Ph.D.

_____ _____

Treating Physician Date
Name: