InfoLEISH

redeLEISH newsletter - 5th edition

October 2021

EDITORIAL

At the beginning of 2020, notable changes were introduced in our way of working and living. Public health programs and research priorities were shifted, and many activities were halted to prioritize actions aimed to manage and control the COVID-19 pandemic.

The medical attention to cutaneous leishmaniasis (CL) patients routinely provided by specialized clinics has been interrupted and, in many cases, patients were abandoned to deal with the disease by themselves.

DND*i*'s CL program has also been affected, resulting in delays, budget restrictions and, in some cases, the suspension of activities. Despite all these constraints and setbacks during the 2020-2021 period, significant milestones were reached.

The Phase III study to assess the combination of thermotherapy and miltefosine started in Panama, where so far nearly half of the required patients have been enrolled. The sites in Brazil and Peru have also started screening and enrolling patients, while in Bolivia the study is expected to start in Q4-2021. At the end of 2020, all the necessary preclinical studies were completed, which allowed the first-in-human study with CpG-D35 to be initiated. A single ascending dose study is currently being conducted in the United Kingdom and is expected to be completed by the end of 2021. We continue with studies aiming to characterize the *in vivo* and *in vitro* activity of the different oral compounds that have been identified to be active against visceral leishmaniasis. We hope that the second half of 2021 and the years ahead will allow us to advance our plans to deliver a new, safe, effective treatment for CL patients, and thus contribute to the World Health Organization NTD roadmap 2021-2030.

Summary

02 Tribute to Moacir Antônio Zini (1972-2020)

03 Efficacy and safety of miltefosine compared to liposomal amphotericin B for the treatment of leishmaniasis

03 Safety and clinical activity of Curaleish in the topical treatment of CL

04 Oral miltefosine in combination with topical paromomycin for ATL

05 Efficacy and safety of single dose intralesional meglumine antimoniate and topical paromomycin gel in the treatment of CL

05 Efficacy and safety of intralesional meglumine antimoniate for the treatment of localized CL

06 Advances in the development of the CpG-D35

07 Background and possibilities of immunotherapy in CL

08 Data sharing project: a successful redeLEISH collaboration

10 Advances in standardization and multicenter validation of real-time PCR assays

11 ECLIPSE: empowering people with CL

13 The process of implementing miltefosine for the treatment of tegumentary leishmaniasis in Brazil

TRIBUTE TO MOACIR ANTÔNIO ZINI (1972-2020)

When Moacir Zini entered the consulting room, in the mid-1990s, infectious disease specialist Marcia Hueb realized that she did not have a trivial story in front of her. The contrast was obvious: on the outside, a body covered in lesions and scars; inside, a tenacity she had rarely seen in a patient.

For almost 30 years, Moacir carried the marks of diffuse cutaneous leishmaniasis, an aggressive type of the disease, with very few cases registered in the world. Born in Catanduvas (Paraná, Brasil), he discovered the disease at a very young age, when he was working as a gold miner. "The doctor told me: 'it would be worse if you had malaria'. Today I see that he was wrong", he recalled, during a lecture at the DND*i* Partners' Meeting in Rio de Janeiro.

The path soon turned out to be more tortuous than anticipated. The first treatment with meglumine antimoniate did not work as expected, which led Moacir to look for new alternatives at the reference center of the Júlio Müller University Hospital, in Cuiabá (MT), where Marcia worked.

"At that time, he lived in Peixoto de Azevedo, in the interior of Mato Grosso. He had already gone through several treatments, including experimental ones. It was up to me to calm him down, as we had more questions than answers," said the doctor.

Moacir mapped scientific events on cutaneous leishmaniasis in his state, in order to approach specialists and, perhaps, discover more effective solutions for his case. That's how he met Ana Nilce Elkhoury, regional advisor for leishmaniasis at the Pan American Health Organization, who invited him to talk with the professionals at the training she was conducting in the capital of Mato Grosso.

"The clarity of his story impressed me, and I decided to take Moacir's case to institutions that developed treatments for the disease, such as DND*i*, to find out what we could do to help him", she explained.

The good response to miltefosine was a glimmer of hope in Moacir's trajectory. The illness had regressed so significantly in the first six months that he was able to go back doing seemingly simple tasks like wearing a pair of shoes. "At our wedding he entered the church barefoot," recalled nurse Talita Zini, his partner of nearly 18 years.

The partnership between the two, by the way, went far beyond matrimonial bonds. Together, Moacir and Talita became activists, creating the Brazilian Association of People with Leishmaniasis (ABRAPleish), the first entity in Brazil aimed at welcoming and guiding people living with the disease. "We've always been side by side. He was very strong, even when affected by the side effects of the treatment", she recalled emotionally.

After a battle of more than 20 years, Moacir died on December 9, 2020. He left two daughters and a multitude of friends in life and in struggle. DND*i* expresses its condolences and, inspired by Moacir, reaffirms its objective of seeking safe, effective and accessible treatments for everyone living with leishmaniasis.

EFFICACY AND SAFETY OF MILTEFOSINE COMPARED TO LIPOSOMAL AMPHOTERICIN B FOR THE TREATMENT OF LEISHMANIASIS

GLAUCIA COTA
Instituto René Rachou, Fundação Oswaldo Cruz, Brazil

A Phase III, open label, randomized and controlled clinical trial (ReBEC RBR-5r93wn) to assess the non-inferiority of the efficacy and safety of a treatment for mucosal leishmaniasis with miltefosine compared to liposomal amphotericin B. This is a multicenter study carried out in Brazil, with a planned total sample size of 110 participants, involving four institutions: René Rachou Institute (Minas Gerais), Júlio Muller University Hospital (Mato Grosso), Emílio Ribas Infectious Disease Institute, and Hospital das Clínicas of the University of São Paulo. The potential advantages of miltefosine are its availability in oral form and potential use by elderly patients or patients with comorbidities. On the other hand, the standard treatment with liposomal amphotericin B is justified due to its better safety profile compared to meglumine antimoniate and the availability of efficacy data in the literature. The compared interventions are miltefosine orally for 28 days (50mg, 2 to 3 times/day), and liposomal amphotericin B (total cumulative dose of 30mg/kg). Aiming to explore an intermittent treatment regimen, a small group of patients will receive a total cumulative dose of 30mg/kg of liposomal amphotericin B divided into three administrations of 10mg/kg every seven days. Outcomes of interest are the cure rate at 90 and 180 days from the start of treatment, recurrence at one year, and the rate and intensity of adverse events in each arm. The study is in the recruitment phase, with approximately 50% of the sample already enrolled, and it is expected to be completed by December 2022. It is expected that this evaluation will gather evidence to support the use of an oral alternative for mucosal leishmaniasis, one of the most morbid and difficult to treat forms of tegumentary leishmaniasis. •

EVALUATION OF THE SAFETY AND CLINICAL ACTIVITY OF CURALEISH FOR TOPICAL TREATMENT OF CUTANEOUS LEISHMANIASIS

LILIANA LÓPEZ, SARA ROBLEDO E IVÁN VÉLEZ
Programa de Estudio y Control de Enfermedades Tropicales (PECET), Facultad de Medicina, Universidad de Antioquia, Colombia.

In response to the WHO's recommendation to focus research efforts on the control of cutaneous leishmaniasis (CL) by searching for new safe, effective and affordable alternative drugs for oral, topical or parenteral administration, with shorter treatment cycles and lower toxicity (1), Curaleish was developed. Curaleish is a topical formulation in lotion and cream (the lotion is hydroalcoholic and the cream is glycolic), containing natural extracts from the branches (stems and leaves) of the tree *Caesalpinia spinosa*, also known as "Tara" or "Davidivi."

To assess the safety and tolerability of Curaleish, a Phase Ib-II clinical trial is planned, involving 50 volunteers with a confirmed diagnosis of uncomplicated CL. They will be randomly assigned to receive three daily applications of lotion and two daily applications of cream for four or six weeks. Volunteers will be monitored for six months.

The study is undergoing the approval process by the Colombian regulatory agency; it is planned to start in Q4-2021, and it is estimated that it will take 18 months to be executed. The identification code in clinicaltrials.gov is NCT04072874. •

References

(1) World Health Organization (WHO). Control of leishmaniasis, in Sixtieth World Health Assembly 2007. [Internet] [Accessed 2015 Nov 16] Available at: http://apps.who.int/gb/ebwha/pdf_files/WHASSA_WHA60-Rec1/E/cover-intro-60- en.pdf

ORAL MILTEFOSINE IN COMBINATION WITH TOPICAL PAROMOMYCIN FOR AMERICAN CUTANEOUS LEISHMANIASIS

JAIME SOTO, Fundación Nacional de Dermatología
and Hospital Dermatológico de Jorochito, Bolivia

In Latin America, any of the systemic antileishmanial drugs cure between 50% and 85% of patients with cutaneous leishmaniasis when used as monotherapy — figures that are far from ideal. In response to the WHO/PAHO recommendation to seek local therapeutic options and drug combinations, in January 2019 we initiated a randomized, double-blind controlled clinical trial comparing three groups with 40 patients each: oral miltefosine plus 15% paromomycin cream vs. oral miltefosine plus vehicle cream vs. 15% paromomycin cream (ClinicalTrials.gov Identifier: NCT03829917).

The patients came from different endemic regions of Bolivia and were admitted to the Dermatological Hospital of Jorochito throughout the treatment. They were subsequently followed-up for six months. The parasitology studies were carried out in the Department of Parasitology of the National University of Colombia, where, in addition to species identification, the researchers attempted to identify the presence of *Leishmania* RNA virus (LRV). A local foundation (Funderma) conducted the project, and The Albert Berman Foundation for Medical Research (ABF) funded it. The National Leishmaniasis Program of the Ministry of Health of Bolivia collaborated with the regulatory aspects and the search and referral of patients, which was coordinated and supported by the regional health districts of the endemic areas. The Bioethics Committee of the Universidad Mayor de San Simón, in Cochabamba, analyzed and authorized the study. Fourteen people worked on this project.

The first visit of the first subject was on February 6, 2019, and the last visit of the last subject was on November 9, 2020. A total of 116 subjects completed the trial, and four were lost to follow-up. All subjects were older than 12 years of age, 91% were male, and 25 out of 120 had previously been treated with meglumine antimoniate, and their treatment had failed. In 45 of 59 isolates, *Leishmania* was cultivated, and in all samples where it was identified (89%), the parasite was *L. brasiliensis*, while in the remaining 11% the species could not be identified. An interesting part of the study involved comparing the results of the clinical outcomes of the subjects according to the scheduled visits that we usually follow in our center (follow-up visits at one, three and six months after the end of treatment) with the calendar of visits recommended by the WHO/PAHO advisory group (follow-up visits at two, four and six months after the start of the treatment).

These are the data that we can comment on up to now, as we are currently in the analysis and processing phase.

What difficulties did we face in this trial? The most significant was the procurement of the medications, because the supply of miltefosine is limited and paromomycin cream is not commercially available. Relying on the group's prior experience with a custom-made paromomycin cream manufactured by a local pharmacy, we prepared the cream and the vehicle for this trial using an API purchased in China and following the original development methodology that is in the public domain, introducing some modifications. This study was planned and executed by the researchers without the participation of the pharmaceutical industry, and the drugs were purchased with the center's own funds.

In previous studies with paromomycin cream at 15% used in monotherapy, the efficacy reached 70%, while the efficacy of miltefosine for cutaneous leishmaniasis has ranged between 71% and 84% in the different studies carried out by the group. It is expected that the simultaneous use in combination of these two drugs will improve efficacy, as has been the case with other combinations we have tested (miltefosine plus intralesional pentamidine, for example), but this is still under analysis.

In Bolivia, as in most countries in the region, we are facing serious challenges due to the loss of effectiveness of antileishmanial drugs and the very limited supply of new molecules, so we must look for ways to enhance existing drugs, improving their efficacy without increasing the risks of potential severe adverse effects.

Current recommendations indicate the use of systemic or local therapies as monotherapy when the patient is treated for the first time, recommending combined therapies for the management of therapeutic failures. In my view, and this is a personal opinion for which I take sole responsibility, combined treatments should be used as first treatment, because the efficacy/effectiveness of any of the monotherapies leaves many cases unresolved, and "with leishmaniasis, the first treatment should be the last." •

EFFICACY AND SAFETY OF SINGLE-DOSE OF INTRALESIONAL MEGLUMINE ANTIMONIATE AND TOPICAL PAROMOMYCIN GEL COMPARED TO INTRALESIONAL APPLICATIONS OF MEGLUMINE ANTIMONIATE FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS

GLAUCIA COTA
Instituto René Rachou, Fundação Oswaldo Cruz, Brazil

Aiming to fill an important gap in the pharmaceutical market, namely the availability of a topical drug for the treatment of skin lesions caused by infection of *Leishmania* parasites, especially the species circulating in the Americas, i.e. *L.* (Viannia) *braziliensis*, *L.* (L.) *amazonensis* and *L.* (V.) *guyanensis*, the Fiocruz Institute of Drug Technology (Farmanguinhos) is working on a plan to develop a topical formulation of paromomycin sulfate (gel). This development stems from scientific efforts initiated at the School of Pharmacy of the Federal University of Minas Gerais (UFMG), which conducted the first experimental development of the gel formulation, subsequently transferring the technology to Oswaldo Cruz Foundation (Fiocruz). The clinical evaluation strategy for the product at this time is to conduct an open-label, randomized Phase III clinical trial (RBR-4ypn69) comparing the efficacy and safety of a sequential single-dose treatment of intralesional meglumine antimoniate and topical gel paromomycin for 30 days, versus a treatment with three intralesional applications of meglumine antimoniate (the treatment recommended by the Ministry of Health) in adult and adolescent patients with cutaneous leishmaniasis. The study is expected to start recruiting in 2022 and to involve three sites in Brazil, with an estimated sample size of 114 participants. The primary endpoint will be defined by complete epithelialization of the lesion and will be analyzed at D90±7 days; adverse events will be monitored through clinical examination, blood count, biochemical testing and electrocardiogram. Our expectation is to be able to offer a Brazilian-made product at lower cost to treat a neglected disease that affects vulnerable populations, a product that can be used at home and, especially, which is less toxic, with the same efficacy as the therapeutic options currently available for the treatment of cutaneous leishmaniasis. •

A MULTICENTER STUDY TO EVALUATE THE EFFICACY AND SAFETY OF INTRALESIONAL ADMINISTRATION OF MEGLUMINE ANTIMONIATE COMPARED TO SYSTEMIC TREATMENT FOR LOCALIZED CUTANEOUS LEISHMANIASIS

MARCELO ROSANDISKI LYRA and MARIA INÊS FERNANDES,
Instituto Nacional de Infectologia Evandro Chagas,
Fundação Oswaldo Cruz, Brazil

This multicenter Phase III clinical trial on the efficacy and safety of intralesional (IL) meglumine antimoniate (MA) compared with systemic administration (S) was conducted in seven Brazilian states. The study is coordinated by the Laboratory of Clinical Research and Surveillance in Leishmaniasis (LaPClinVigiLeish), of the National Institute of Infectious Diseases Evandro Chagas (INI/Fiocruz), with collaborators from the universities of São Paulo (USP), Mato Grosso (UFMT), Mato Grosso do Sul (UFMS), Brasilia (UnB), in addition to the René Rachou Institute (CPQRR/Fiocruz) and the Dr. Heitor Vieira Dourado Tropical Medicine Foundation. It is supported by CNPq/Fiocruz and the Pan American Health Organization (PAHO). We have expanded IL recommendations for up to three lesions measuring up to 5cm, on joints, and with a maximum of 15ml of MA per day. We performed three IL infiltrations with a14-day interval. During the treatment, the patients were assessed every seven days (S) or every 15 days (IL), and on days 45, 90, 180, 360 and 720. Outcomes: Initial cure/secondary outcome at D90 ± 7 days was complete epithelialization (ulcerated lesions); definitive cure/primary outcome at D180 ± 14 days was complete epithelialization and total involution of infiltration (nodules, plaques and ulcerations). Therapeutic failure: non-epithelialized/non-healed lesion on those dates. Adverse events were categorized into clinical, laboratory and ECG, monitored through clinical examination, standard questionnaire, laboratory tests and periodic ECG evaluations, with regards to frequency, intensity and causality with the treatment. A total of 135 participants were included. The cure rate of IL was 70.6% [CI=0.583-0.810] per intention- to-treat and 82.8% [CI=0.705-0.914] in the per protocol analysis, in the S it was 59.7% [CI= 0.470-0.715] and 67.8% [CI=0.533-0.783] respectively. Eleven participants discontinued treatment due to AE (10S and 1IL). IL treatment proved to be non-inferior and less toxic than S. •

ADVANCES IN THE DEVELOPMENT OF CPG-D35

BYRON ARANA, DND*i*

CpG ODN D35 is a class A CpG ODN TLR9 agonist which stimulates maturation and activation of plasmacytoid dendritic cells and production of pro-inflammatory cytokines such as IFN-α and IFN-γ, but has little or no effect on B cells and does not foster the Th2 type response.

Given its properties, CpG ODN D35 in combination with chemotherapy has the potential to significantly improve the treatment of patients with complicated CL forms, and for this reason it was included in the DND*i* portfolio for its development.

The pharmaceutical development studies have demonstrated the suitability of the drug product to be used in the first in-human studies. All the non-clinical studies were completed during 2020, and their results demonstrated that the compound is safe and well tolerated at any of the three tested doses, and the systemic exposure is dose-proportional. No clinical signs or target organ toxicities were identified in the toxicological studies. Local changes at the injection site such as oedema were reported at all doses tested, but were expected and reversible given the nature of CpG ODN D35.

DND*i* is now initiating a Phase I, single ascending study (SAD) to investigate the safety, tolerability, pharmacokinetics, and immunoreactivity of CpG-D35 in healthy subjects. The study is being conducted in the UK and the enrollment of subjects initiated in June 2021. This study will bring crucial information to evaluate if further development of this compound will be possible.

Results of the SAD study are expected to be available by Q1-2022. If the compound is shown to be safe, tolerable and to elicit the expected immune response, we will continue with a multiple ascending dose study (MAD) next year in subjects with *Leishmania* lesions. •

BACKGROUND AND POSSIBILITIES OF IMMUNOTHERAPY FOR CUTANEOUS LEISHMANIASIS

PAULO MACHADO, Universidade Federal da Bahia, Brazil

The use of combined drugs for the treatment of diseases caused by intracellular agents has long been indicated for tuberculosis and leprosy, with the goal of increasing the therapeutic response and reducing the chances of causing drug resistance. In the case of cutaneous leishmaniasis (CL), conventional therapeutic regimens use a single drug, notably pentavalent antimonial (Sb^v), which is the most commonly used drug in most endemic countries since 1945. However, the increasing rates of therapeutic failure, its high toxicity, and its exclusively parenteral administration are factors that call for other medications to replace it, or for associations that can increase its efficacy while decreasing the dosage and toxicity.

Among the possible strategies for therapeutic associations, the use of immunomodulators is based on the understanding that tissue injury in CL is the result not only of parasite action, but largely of the intense and exaggerated immunological and inflammatory response, which also contributes to the reduction of tissue repair processes, causing the ulcers to take longer to heal. For example, the ulcer size in patients with CL is directly related to T-cell activation, interferon (IFN) production and the tissue necrosis factor (TNF). Previous studies by our group have shown that the granulocyte-macrophage colony-stimulating factor cytokine (GM-CSF) in association with Sb^v, both subcutaneously and topically (occlusive dressings), was more effective than Sb^v and placebo, increasing the cure rate and accelerating healing. More recently, miltefosine associated with the topical use of a cream containing 0.1% GM-CSF (M+GM group) was compared to the conventional treatment with Sb^v (Sb^v group) and to miltefosine with a topical cream vehicle (M+P group) in patients with CL caused by *L. braziliensis*. A total of 133 patients were included, and the final results showed a cure rate of 76% for the M+GM group, 77% for M+P, and only 44% for the Sb^v group in cases caused by *L. braziliensis*, as well as shorter healing time in the two groups that used miltefosine. The same study with 150 patients with CL by *L. guyanensis* showed lower cure rates with miltefosine: 58% for the M+GM group, 66% for M+P, and 52% for the Sb^v group. In these two studies, the cream with 0.1% GM-CSF showed no adjuvant effect, but miltefosine had confirmed superiority in cases by *L. braziliensis* or a similar cure rate in those caused by *L. guyanensis*.

It is concerning that the best cure rate of miltefosine remains below 80%, which stresses the importance of seeking alternatives for new associations. One of these alternatives is another cytokine, the granulocyte colony-stimulating factor (G-CSF), which helps inhibit the action of cytotoxic CD8 T cells, implicated in the pathogenesis of CL, induces the production of IL-10, which is anti-inflammatory, and stimulates skin healing in toxic epidermal necrolysis and in epidermolysis bullosa. We are currently conducting a randomized, double-blind clinical trial in Corte de Pedra, in Bahia (Brazil), comparing the intralesional use of G-CSF in association with Sb^v versus conventional monotherapy in patients with CL caused by *L. braziliensis*. •

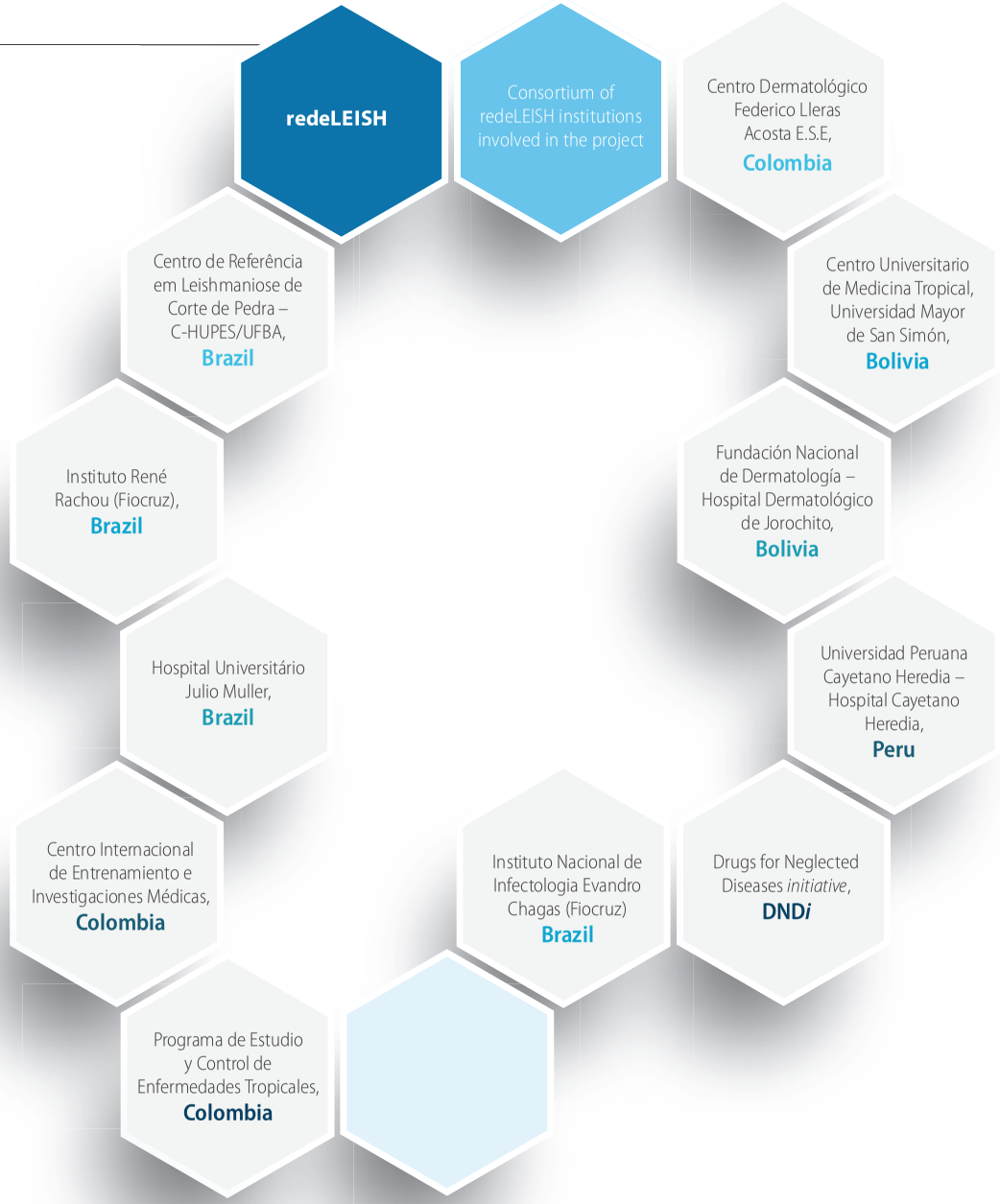
DATA SHARING PROJECT: A SUCCESSFUL redeLEISH COLLABORATION

During the redeLEISH annual meeting in 2018, investigators proposed to join efforts seeking to share information collected by cutaneous leishmaniasis (CL) research centers in the region about how children ≤ 10 years of age and adults ≥ 60 years of age are being treated and their response to treatment. These special population groups are of particular interest because they are usually not included in clinical trials for ethical and safety concerns, and therefore specific treatment guidance is uncertain due to the scarceness of robust evidence. The purpose of the proposal to the participant centers was to share key data from their databases with the aim of describing the effectiveness and tolerability of routine anti-leishmanial treatments, with the ultimate goal of providing recommendations to the national leishmaniasis programs in Latin America for improved management of these CL patients.

As shown in the schematic representation of the consortium, 11 redeLEISH institutions collaborated in the project. TDR/WHO provided funds to organize the data collection and analysis. Governance of the collaboration was guaranteed through monthly teleconferences allowing the group to discuss and, together, build all project documents, such as study protocol – approved by all of the respective institutional Ethical Committees, – data base, statistical analysis plan, analyses, and final report. Though the COVID-19 pandemic delayed its execution, the contingency plan established by the group, as well as the commitment and efforts of all of its participants allowed the successful completion of the project in March 2021.

With data from 1,325 CL patients (736 children ≤ 10 years of age and 589 adults ≥ 60 years of age) treated between 2014 and 2018 in the ten participant sites in four countries, this is, to our knowledge, the largest collaborative study of this type for CL in the region.

One of the lessons learnt in this project is that follow-up of patients after initiating antileishmanial treatments remains a significant challenge in the region: lack of information on clinical response was found to be the main reason for exclusion, and



data analysis showed that a low number of patients, especially children, had follow-up information for two post-treatment visits. This underscores the need to develop strategies for improving patient follow-up, with special attention to the pediatric population. As an indirect result of this project, some groups are re-organizing their patient management activities to improve follow-up of patients.

Another key finding that emerged is the need to increase and implement the use and access to alternative treatment options, such as local therapies (thermotherapy, intralesional antimonials), miltefosine, and liposomal amphotericin B, particularly in older patients. This regional study documents that systemic antimonials are still widely used in these special populations, despite the known toxicity, contraindications in elderly patients, and long duration of treatment resulting in a social, logistic and financial burden to both patients and health care providers. The use of treatments other than antimonials will help to achieve the "critical action 1 for CL", of the World Health Organization's NTD roadmap 2021-2030 roadmap, which aims to "develop and scale-up easy-to-administer oral or topical treatments that could be used in health centers".

We are now coordinating the dissemination of the results of this productive and successful collaboration that encourages other regional initiatives to effectively address important unmet needs through similar regional inter-institutional cooperation. •

ADVANCES IN THE MULTICENTER STANDARDIZATION AND VALIDATION OF CONSENSUS REAL-TIME PCR ASSAYS FOR THE MOLECULAR DIAGNOSIS OF CUTANEOUS LEISHMANIASIS IN THE AMERICAS

OTACÍLIO C. MOREIRA[1]; ANA NILCE SILVEIRA ELKHOURY[2] and ELISA CUPOLILLO[3]
[1]Laboratório de Biologia Molecular e Doenças Endêmicas, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Brazil
[2]Pan-American Health Organization, Brazil
[3]Laboratório de Pesquisa em Leishmanioses, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Brazil

In the last two decades, approximately one million cases of tegumentary leishmaniasis (TL) have been registered in the Americas, with cutaneous leishmaniasis (CL) being the most common manifestation in most affected areas (PAHO/WHO, 2020). Different *Leishmania* species circulate in the region and cause the disease in humans, with *Leishmania braziliensis* being the most dispersed, but other species also have epidemiological importance depending on the geographic region (PAHO/WHO, 2019).

The clinical suspicion of TL is confirmed by direct parasitological tests, mainly through microscopic visualization of the parasite in material collected from the lesions. These tests have high specificity, but low sensitivity, which has driven the search for new methods for diagnosing the disease. Since the first work demonstrating the usefulness of PCR for the diagnosis of CL, several methodologies have been proposed and tested, whether conventional PCR (cPCR) or quantitative real-time PCR (qPCR). However, there have been no efforts to standardize and validate a methodology for the diagnosis of CL, making it difficult to reach a consensus regarding protocols and molecular targets that can be used to assist in clinical diagnosis.

In this context, the Pan American Health Organization (PAHO/WHO) is coordinating a multicenter project involving different research groups from CL-endemic countries with the objective of proposing a standardized and validated consensus methodology for the parasitological diagnosis of the disease by qPCR. Seven groups are participating in the project, from Argentina, Bolivia, Mexico, Panama, Peru and two from Brazil.

The qPCR standardization assays were conducted with DNA extracted from promastigotes of different *Leishmania* species, followed by a preliminary clinical validation with samples collected from patients clinically suspected of having CL and with a confirmed or unconfirmed parasitological diagnosis. Multiplex qPCR assays targeting human RNAse P, and 18S rDNA and HSP70 in the parasite, were performed. A good correlation was observed with respect to the quantification of parasites in the two targets used. Considering microscopic examination as the gold standard for parasitological diagnosis, we obtained a sensitivity of 98.5% in the simultaneous analysis of the two qPCR assay results. Furthermore, the maximum specificity (100%) was obtained for the sequential analysis of the results of qPCR for 18S rDNA, followed by qPCR for HSP70 only for samples which were negative for the first target (Filgueira et al., 2020).

As the last stage of the study, a multicenter clinical validation of the standardized methodology is being carried out. At least 15 samples of skin lesions from patients with CL were collected by each of the participating laboratories. The DNA of each sample was extracted and sent to Oswaldo Cruz Foundation (Fiocruz), in Rio de Janeiro, where they are being submitted to qPCR tests. A panel of samples will then be built to be sent blindly to each group to perform the tests and concordance analysis of the results. In the end, we hope to have available a methodology which is a consensus among experts, duly standardized and validated in a multicentric way, to be applied as an auxiliary tool for the diagnosis of CL in the Americas.

All scientific and technical activities are being coordinated by the Leishmaniasis Research Laboratory and the RPT09A Real Time PCR Platform – Laboratory of Molecular Biology and Endemic Diseases (Fiocruz) with support from PAHO/WHO and DND*i*. •

References

Pan-American Health Organization. Manual of procedures for leishmaniasis surveillance and control in the Americas Washington, D.C.: OPS; 2019. Available at https://iris.paho.org/handle/10665.2/51838

Pan-American Health Organization. Leishmaniasis. Epidemiological Report of the Americas. Number 9, December 2020. Washungton DC. PAHO.; 2020. Available at: https://iris.paho.org/handle/10665.2/53090

Filgueira CPB, Moreira OC, Cantanhêde LM, de Farias HMT, Porrozzi R, Britto C, et al. (2020) Comparison and clinical validation of qPCR assays targeting Leishmania 18S rDNA and HSP70 genes in patients with American Tegumentary Leishmaniasis. PLoS Negl Trop Dis 14(10): e0008750. https://doi.org/10.1371/journal.pntd.0008750

ECLIPSE: EMPOWERING PEOPLE WITH CUTANEOUS LEISHMANIASIS

SUNETH AGAMPODI[1], LISA DIKOMITIS[2], PAULO MACHADO[3], AFEWORK MULUGETA[4], HELEN PRICE[2], LENY TRAD[3] on behalf of all ECLIPSE team members
[1]Rajarata University, Sri Lanka
[2]Keele University, United Kingdom
[3]Universidade Federal da Bahia, Brazil
[4]Mekelle University, Ethiopia

FUNDED BY NIHR | National Institute for Health Research
UKaid from the British people

ECLIPSE (Empowering people with cutaneous leishmaniasis: Intervention programme to improve patient journey and reduce stigma via community education) is a four-year global health programme co-led by Professor Lisa Dikomitis and Dr Helen Price at Keele University (UK). ECLIPSE is funded by the UK's National Institute for Health Research (NIHR).

ECLIPSE brings together an international, cross-cultural and multidisciplinary team from Keele University (UK), a team from the Federal University of Bahia (Brazil) led by Professor Leny Trad and Dr Paulo Machado, a team from Mekelle University (Ethiopia) led by Professor Afework Mulugeta and a team from Rajarata University of Sri Lanka, led by Professor Suneth Agampodi. This four-country ECLIPSE team comprises clinicians, anthropologists, psychologists, parasitologists, leishmaniasis and public health specialists. The ECLIPSE partnership includes both senior research leaders and a large cohort of early career researchers. The ECLIPSE Policy Network brings together policymakers from Brazil, Ethiopia and Sri Lanka.

ECLIPSE researchers are using a range of qualitative and quantitative social science research methods to gain an in-depth understanding of the experiences, understandings and perceptions of people with cutaneous leishmaniasis (CL), community members and healthcare professionals, and to measure CL awareness and stigma. These insights will inform the development of the ECLIPSE community-based interventions and a training package for healthcare workers.

- **EXAMINE CUTANEOUS LEISHMANIASIS IN CONTEXT** (social, cultural, economic, health system and political context in CL-endemic communities)
- **IMPROVE MENTAL AND PHYSICAL HEALTH OUTCOMES OF PEOPLE WITH CL**
- **CO-PRODUCE CONTEXT BESPOKE COMMUNITY-BASED INTERVENTIONS**
- **DEVELOP TAILORED TRAINING PACKAGES FOR LOCAL HEALTHCARE WORKERS**
- **THE ECLIPSE POLICY NETWORK WILL SHARE BEST PRATICE AND ACCELERATE DISSEMINATION**
- **ENHANCE RESEARCH CAPACITY IN APPLIED HEALTH RESEARCH AT ALL LEVELS**

ECLIPSE is in the community. Indeed, ECLIPSE team members will not conduct any research or public health interventions without input from residents in the rural, and often remote, CL-affected communities in Brazil, Ethiopia and Sri Lanka. Therefore, robust community engagement and involvement (CEI) underpins all aspects of ECLIPSE. This ensures that ECLIPSE activities are ethical, effective and appropriately oriented to the needs of people with CL and the wider community. CEI is different in each ECLIPSE country, because CEI activities are culturally adapted and bespoke to their specific contexts. The objective is the same: to collaborate with community members. This reflects the ethos of ECLIPSE:

"No research about us without us."

The ECLIPSE CEI strategy is based on the establishment of two groups: (a) community advisory groups (CAGs) and (b) community of practices (CoPs). CAGs are situated at a community level, comprising mostly of people with CL and community members, while CoPs are based at an urban regional level and are attended by a wide range of stakeholders (including clinicians, public health officials, policymakers, subject experts). The role of CAG and CoP members is to provide input on different aspects of the project, such as public-facing material, participant recruitment processes, interpretation of study findings, development and co-production of interventions, dissemination of project findings and acceleration and implementation of new knowledge implications for practice. •

More information

www.eclipse-community.com, @ECLIPSE_Keele and eclipse.community@keele.ac.uk

THE PROCESS OF IMPLEMENTING MILTEFOSINE FOR THE TREATMENT OF TEGUMENTARY LEISHMANIASIS IN BRAZIL

KATHIELY MARTINS DOS SANTOS,
LUCAS EDEL DONATO, MARCIA LEITE DE SOUSA GOMES,
GT-Leishmanioses
Coordenação-Geral de Vigilância de Zoonoses e Doenças de Transmissão Vetorial – CGZV
Departamento de Imunizações e Doenças Transmissíveis – DEIDT
Secretaria de Vigilância em Saúde - Ministério da Saúde (SVS/MS), Brazil

The trajectory of the incorporation of miltefosine for the treatment of tegumentary leishmaniasis (TL) in Brazil dates to approximately ten years ago, when in 2010, the then Commission for the Incorporation of Technologies of the Ministry of Health (CITEC) decided to incorporate the drug into the Brazilian National Health System (SUS). That same year, the World Health Organization (WHO) determined the inclusion of miltefosine in the List of Essential Medicines, recommending that countries' regulatory bodies prioritize this product within the public health systems.

Then, Law No. 12401 of April 28, 2011 came into force in the country, regulating therapeutic care and the incorporation of health technology in the scope of the SUS. The enactment of this law vetoed, in all spheres of SUS management, the dispensing, payment, compensation or reimbursement of national or imported medications and products without registration at the National Health Regulatory Agency (Anvisa). Furthermore, the aforementioned law created the National Commission for the Incorporation of Technologies in the SUS (CONITEC), replacing CITEC. The regulation of the directives of this new commission is supported by Decree No. 7.646/11, which has the effect of regulating the composition and powers of the Commission.

Finally, the creation of the regulation imposed by the aforementioned law for the incorporation of technology in the SUS meant that, at that time,

as far as CITEC's decision was concerned, the incorporation of miltefosine in the SUS did not materialize.

In 2016, the Technical Group on Leishmaniasis of the Health Surveillance Secretariat of the Ministry of Health resumed the agenda for incorporating miltefosine, requesting that CONITEC reconsider the claim.

On November 10, 2016, the CONITEC Plenary unanimously recommended the incorporation of miltefosine into the SUS for the treatment of people affected by tegumentary leishmaniasis, taking the issue to Public Consultation No. 40, from which six technical contributions were derived, along with another three on experience or opinion, none of which influenced the merits of the preliminary recommendation.

Notwithstanding the unanimous recommendation, validated by the result of the Public Consultation, the obstacle brought by the legal framework persisted in the path of miltefosine's incorporation. This was resolved by Opinion No. 00573/2017 of the Legal Consultancy (Conjur) with the Ministry of Health, which — restricted to the legal aspects — concluded that CONITEC had the

legal authority to decide on medicines without registration with Anvisa, under the terms of Law No. 9.782 of January 26, 1999, which exempts the registration of medicines and other strategic supplies when acquired through international multilateral organizations and for use in public health programs by the Ministry of Health and its related entities.

Ordinance No. 56 of October 30, 2018 made public the decision to incorporate miltefosine for the first-line treatment of tegumentary leishmaniasis within the scope of the SUS. Until its incorporation, the available therapeutic alternatives were exclusively for parenteral use, and, although effective, limiting factors, such as the narrow therapeutic window, the length of treatment and the need for assistance at the outpatient or hospital level, linked to the social vulnerability of the population more susceptible to the disease, favored a high rate of non-compliance with therapy, in addition to adverse events caused by the available medications and a higher risk of death.

The guarantee of the first oral-use treatment for TL in the SUS is therefore a response to efforts so that more Brazilians can be assisted and treated safely and effectively, with less invasive, more accessible approaches that promote adherence to the treatment.

Ordinance No. 3.047 of November 28, 2019 included miltefosine in Annex II of the National List of Essential Medicines (Rename, 2020), attributing the competence of its financing, acquisition and distribution to the states and Federal District, and to the Ministry of Health through the Strategic Component of Pharmaceutical Assistance.

Also in the list of regulatory aspects and in view of the potential for teratogenesis, in Brazil miltefosine was included in List C1 of Ordinance No. 344 of May 12, 1998, which approves the technical regulation on substances and medicines subject to special control.

The Anvisa Resolution RDC No. 337, of February 11, 2020, which deals with the classification of miltefosine in the special control Ordinance, established national criteria for the prescription, dispensing and use by patients of childbearing age, also including health aspects related to the medicine's label and package insert, among others.

Based on this resolution and considering the available evidence, guidelines on the use of miltefosine for the treatment of TL in the SUS, as well as on its monitoring and control, were set out in Informative Note No. 13/2020-CGZV/DEIDT/SVS/MS.

Currently, in the Brazilian public health network, miltefosine is indicated for the first-line treatment of TL, and its therapeutic use is cautioned for the mucosal form of the disease, advising that in these cases, the therapy should be assessed by a specialist physician. The same applies to children weighing less than 30 kg, patients of childbearing age with the possibility of pregnancy and people living with co-infection *Leishmania*/HIV, for whom the use of miltefosine is possible only when the failure of the conventional treatment is characterized.

The accepted dosage schedule recommends the administration of 2.5 mg/kg/day of miltefosine 50 mg, orally, divided into 2 to 3 daily doses, up to a limit of 150 mg/day (3 capsules/day) for 28 days. It is important to emphasize that doses should be administered with meals, aiming to reduce undesirable gastrointestinal effects, especially nausea and vomiting. For patients weighing between 30 and 45 kg, the recommended daily dose is 100 mg (2 capsules/day).

The treatment is carried out in two stages of 14 days each, with returns to the health service for evaluation on the 13th and 28th days of treatment. This proposed flow aims to ensure patient safety, providing opportunities for monitoring during treatment and at the end of it, so that their evolution and adherence are monitored and the risks of administration error are mitigated.

Another aspect considered in the process of structuring the implementation of treatment in the public health network was surplus and disposal. Due to the framework of Ordinance No. 344/1998, the fractionation of the miltefosine based medication is vetoed to health services; thus, the strategy adopted was national

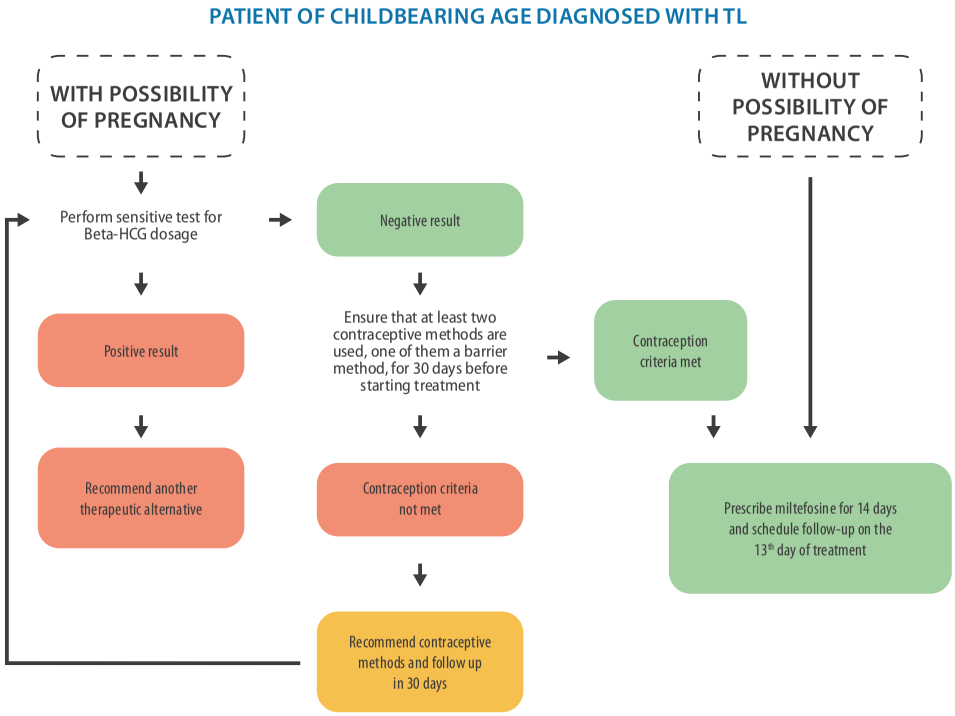


Figure 1: Flow for therapeutic indication of miltefosine in the treatment of patients of childbearing age diagnosed with LT.

repackaging. Through a partnership between the Ministry of Health and the Farmanguinhos laboratory of the Oswaldo Cruz Foundation (Fiocruz), the blisters were repackaged into secondary packages containing 42 capsules each, which allows each patient to receive exactly the amount needed for their 14-day treatment (corresponding to one stage).

However, it was anticipated that there would be a possibility of surplus medication in cases where the therapeutic scheme was restricted to the use of two capsules daily. For these cases, the Ministry of Health adopted the Return Term. During dispensing, the patient and pharmacist sign the document where they both undertake to ensure the return or collection of any leftovers, so that the health services can provide proper disposal. The leftovers of the medication are checked by the services at each medical visit during treatment.

Patients of reproductive age with the possibility of pregnancy can use miltefosine as long as the precautions described in Figure 1 are taken into account. The category "patient of childbearing age" falls between menarche and menopause (first and last menses, respectively).

The sensitive test for Beta HCG dosage is performed immediately at the beginning of treatment and repeated monthly, until four months after completion or interruption of treatment. For patients with irregular menstrual cycles, the pregnancy test is performed every two weeks, until four months after completion or interruption of treatment.

It is recommended that the dosage of Beta HCG be determined within 24 hours before the start of treatment. Beyond this time period, the result is considered inappropriate, and, for safety reasons, it is recommended that it be repeated.

For patients who have undergone a definitive sterilization procedure or confirmed menopause for at least two years, the requirements listed above do not apply.

In the event of pregnancy during treatment, the use of miltefosine must be immediately suspended and the appropriate entities notified of the occurrence.

After the entire trajectory for the incorporation of miltefosine, the Brazilian public health system is currently experiencing the first months of guaranteeing the supply of the medicine. It is a milestone celebrated by all parties involved and especially by SUS users, who now have available the first oral treatment in the context of TL.

Various countries face problems related to access to miltefosine, especially regarding price and commercial availability. Today, the SUS has the capacity to offer miltefosine to about 15% of known TL cases, and it is expected that this capacity will be expanded to 25% in the next year.

The medication is available throughout Brazil and, in this first stage of technology implementation, access is restricted to referral services. The next steps will be focused on the qualification, expansion and sustainability of access to this technology in the SUS. •

ACKNOWLEDGMENTS:

Editorial Board:
Byron Arana, Joelle Rode and Marina Certo

Translation:
Carolina Alfaro and Scriba Traduções

Photos:
Fabio Nascimento, Mariana Abdalla and Vinicius Berger

Coordination:
Joelle Rode

Copyediting:
Carolina Alfaro, Joelle Rode, Karla Menezes and Marina Certo

Design:
Alerta!design

Production:
Karla Menezes and Marina Certo

DNDi
Drugs for Neglected Diseases *initiative*
Iniciativa Medicamentos para Enfermedades Olvidadas
Iniciativa Medicamentos para Doenças Negligenciadas

DND*i* América Latina
Rua São José 70, sala 601- CEP 20010-020 - Rio de Janeiro - Brasil
Tel: +55 21 2529-0426 | www.dndial.org

Sede DND*i*
15 Chemin Camille-Vidart 1202 - Geneva - Switzerland
Tel: +41 22 906 9230 | Fax: +41 22 906 9231 | www.dndi.org