

InfoLEISH

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July 2022

EDITORIAL

As the old saying goes, “Nothing lasts forever”, and as we enter the second half of 2022 we are seeing that many of the restrictions imposed by the COVID-19 pandemic are beginning to be lifted. Some things will never be the same, but what we have learned during the last two years will surely help us to face the new challenges of the future.

The CL program at DNDi has continued advancing, and during the last twelve months several milestones have been achieved. The CpG-D35 single ascending dose study, carried out in healthy volunteers in the UK, was completed in November of 2021. Based on the preliminary results indicating that CPG-D35 is safe and elicits the expected immunological effects, we are now preparing the multiple ascending dose study, which is planned to start by Q4-2022.

To further leverage DNDi's rich and diverse NCE portfolio for leishmaniasis, a three-year project was initiated in late 2021 aiming to better understand the pharmacokinetics and pharmacodynamics in skin of the most advanced oral NCEs identified for VL. This will help generate pre-clinical knowledge with high translational value and will increase the probability of a successful advancement of such candidates into clinical trials for CL.

We continue advocating for the replacement of systemic antimonials for the treatment of uncomplicated CL cases; so, besides continuing with the Phase III study to assess the combination of thermotherapy and miltefosine in Panama, Peru, Brazil and Bolivia, we are also supporting our partners' initiatives to expand the use of local interventions for uncomplicated CL.

Finally, yet importantly, I believe all “Leishmaniacs” are thrilled with the opportunity to get together in Cartagena de Indias, Colombia, to attend the long awaited WL7. Hope to see you all there.

BYRON ARANA, DNDi





Summary

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| <p>01 Editorial</p> <p>02 redeLEISH: Achievements, Perspectives, and Challenges in Promoting Collaborative Research for Cutaneous Leishmaniasis</p> <p>04 Update of PAHO Guideline for the Treatment of Leishmaniasis in the Americas: Adoption Perspectives and Challenges</p> <p>07 Prospective Study of Local Interventions for the Treatment of Uncomplicated Cutaneous Leishmaniasis</p> <p>08 Local Treatments for Cutaneous Leishmaniasis in Ethiopia: from Research to Access</p> | <p>09 First-in-Human Study Testing CpG ODN D35, a Toll-like Receptor 9 Agonist, Proves its Safety and Immune Effect in Healthy Volunteers</p> <p>10 Current Overview and Perspectives in the Development of Diagnostic Tests for Cutaneous Leishmaniasis</p> <p>12 Pediatric Treatment: An Unmet Need</p> <p>13 Paromomycin Gel for the Treatment of Cutaneous Leishmaniasis: the Challenges Behind the Development of a Pharmaceutical Product by Brazilian Public Institutions</p> <p>15 Building a Pathway for Translational Drug Development of Oral Treatments for Cutaneous Leishmaniasis</p> |
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REDELEISH: ACHIEVEMENTS, PERSPECTIVES, AND CHALLENGES IN PROMOTING COLLABORATIVE RESEARCH FOR CUTANEOUS LEISHMANIASIS

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Research networks incorporating different actors, including medical and scientific experts, public and private research/academic institutions, government representatives, and international organizations are part of a worldwide trend to increase regional and international collaborations and bolster coordinated research. redeLEISH is part of this landscape and one of DNDi's activities aiming to reinforce existing clinical research capacities in endemic countries and develop new treatments adapted to the needs of neglected patients. The involvement of experts and institutions from endemic countries guarantees that priorities in research are centered on patient's needs, contributing to a major public health impact.

The scope of redeLEISH is to support the implementation of clinical trials for the evaluation of new therapeutic tools for leishmaniasis, promote

exchange of technical and scientific information, support the implementation of collaborative research projects, facilitate consensus on research priorities and the harmonization of clinical trial design, and encourage debates on research and development challenges in leishmaniasis and strategies to ensure the public health impact of the new treatment options developed.

Coordinated by DNDi, redeLEISH's activities are guided by a technical committee composed by representatives from partner institutions in Latin America, with the PAHO/WHO regional advisor for leishmaniasis as permanent member. This committee provides scientific advice and recommendations regarding regional needs and priorities. The main tools for communication and interaction among redeLEISH members are the virtual web forum, the annual newsletter and the periodic meetings.

Since its creation in 2014, redeLEISH has expanded and now constitutes an effective network of cutaneous leishmaniasis (CL) specialists and research centers, having established itself as a force for the implementation of clinical trials and collaborative projects in the region. It has played an important role in the conduction of the phase II and phase III trials to assess the efficacy and safety of the combination of thermotherapy and miltefosine for uncomplicated CL, as well as in the clinical trial to evaluate the efficacy and safety of miltefosine and liposomal amphotericin B for the treatment of mucosal leishmaniasis (ML). The latter is a collaborative project involving five redeLEISH institutions which emerged as a repercussion of the identification of ML as a research priority and the launching, during the fourth redeLEISH meeting at WorldLeish6 Congress, of the Manifesto letter supporting research in ML, endorsed by redeLEISH members and the Brazilian Society of Tropical Medicine¹.

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¹CARVALHO, E., LLANOS-CUENTAS, A., ROMERO, G. Mucosal leishmaniasis: urgent need for more research. Rev. Soc. Bras. Med. Trop. vol.51 no.1 Uberaba Jan./Feb. 2018



Promoting collaborative research has been an important aspect of the network since its creation. In 2014, the first collaborative research project was launched, aiming to identify the diversity of *Leishmania* species in clinical samples of CL patients in four CL reference centers in Brazil. But the main redeLEISH achievement over the years was the successful conduction and completion of a data sharing project aiming to describe the effectiveness and tolerability of routine anti-leishmanial treatments in children ≤ 10 years of age and adults ≥ 60 years of age. This collaborative project, described in the previous edition of InfoLEISH, involved a consortium of eleven redeLEISH institutions from Brazil, Colombia, Bolivia, and Peru, and was proposed by investigators during one of the network's annual meetings. This fruitful cooperation paves the way for the implementation of further collaborative projects within redeLEISH for diagnosis and treatment of CL patients, according to identified priorities.

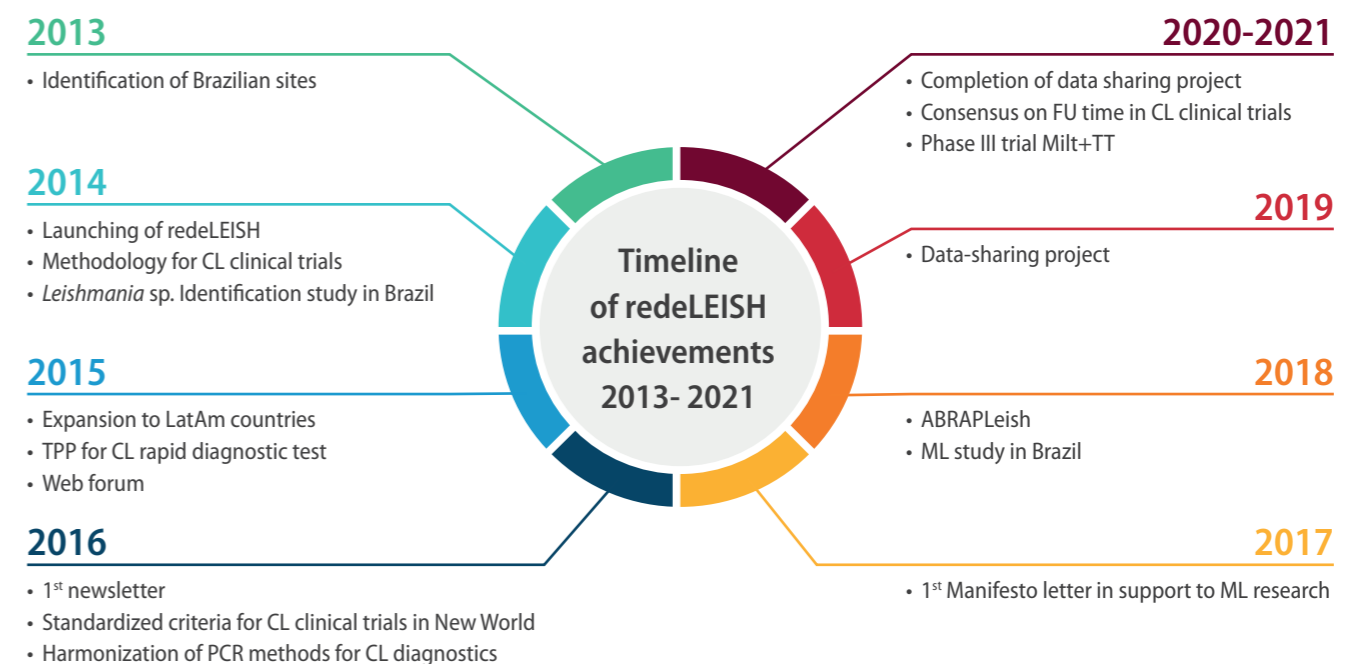
The network has also contributed to the efforts to standardize criteria for conducting clinical trials in CL, in col-

laboration with TDR/WHO², that are now widely endorsed and applied by redeLEISH investigators. In 2021, based on the results of a literature review, experts and investigators from the New World and the Old World reached a consensus recommending the end of patient follow-up in clinical trials assessing treatment efficacy in CL within a range of 90–120 days from treatment start. This recommendation will result in more efficient clinical trials that will benefit patients, drug developers and policy makers. redeLEISH will continue its efforts to expand the adoption of standardized methodologies for conducting clinical trials on CL, an essential requirement for the incorporation of therapeutic innovations and to guide evidence-based treatment recommendations.

In the coming years, the aim is to maintain and increase the engagement of investigators and collaborators in Latin America and attract researchers from the Old World. One of the challenges will be adapting the network's communication tools, particularly the virtual web forum, to increase mem-

ber participation and make discussions more dynamic and relevant. In-person annual meetings have also been impacted by the COVID-19 pandemic, the last one being held in 2019. Though *ad-hoc* virtual meetings have been held and will continue to occur, face-to-face encounters need to be maintained, since key redeLEISH initiatives emerged from these meetings.

In conclusion, thanks to the participation of all its members, redeLEISH has now reached a stage of maturity, increasingly establishing itself as a driving force to encourage collaborative projects and implement clinical trials for leishmaniasis which comply with international standards of quality and ethics in research. The network will be essential for the development of the new chemical entities arriving at the clinical phase and for any research group which is developing new treatments for CL/ML. It will enable new therapeutic options to be made available to the patients in a coordinated way, maximizing competencies and the few resources available for this neglected disease. •



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UPDATE OF PAHO GUIDELINE FOR THE TREATMENT OF LEISHMANIASIS IN THE AMERICAS: ADOPTION PERSPECTIVES AND CHALLENGES

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Leishmaniasis are neglected diseases that mostly affect the poorest people with less access to health care services¹. In the Americas, they are caused by several species of *Leishmania*, which influence the occurrence of various clinical manifestations ranging from benign and self-contained to more severe forms, and impact the response to treatment²⁻⁷. Around 45,000 new cases of cutaneous and mucosal leishmaniasis (CL and ML) and 3,000 cases of visceral leishmaniasis (VL) are reported each year, distributed in 18 and 13 endemic countries, respectively. In 2022, an update to the guideline for the treatment of leishmaniasis in the Americas was published based on the evidence available in the region, with the aim of providing recommendations for the treatment of leishmaniasis in its different clinical forms⁸.

The methodology used to develop this guideline is based on the WHO Handbook, ensuring the transparency of the link between the evidence base and the recommendations. The process covered

from planning to implementation and dissemination, including questions formulated according to the PICO model (population, intervention, comparison and outcome), systematic review, and the formulation of recommendations based on the grading of recommendation assessment, development and evaluation (GRADE methodology)⁹. Moreover, it considers the balance between the certainty of the evidence, the risk-benefit ratio, values and preferences, resource implications, the feasibility of implementing the intervention, the impact on equity, and stakeholder acceptability. When no studies were found to support the recommendations, we updated the best practices from the previous guideline and supplemented them with discussions in the group of participating experts supported by the safety profile of the drugs, studies in other populations and clinical experience managing patients with leishmaniasis.

The recommendations are shown in Tables 1 and 2 according to the clinical form of leishmaniasis, stratified ac-

ording to the grading of evidence and strength of the recommendation. The main advances are for the cutaneous form of the disease, because the updated recommendations are presented according to the age group of the patients and the *Leishmania* species presumably involved. However, the identification of the *Leishmania* species is not imperative to start the treatment. Once the most prevalent species in the endemic area is known, treatment should be initiated according to the clinical status, availability of the drug and the risk-benefit ratio. Other advances for CL include the indication of strong recommendation for the use of local treatments in specific groups of patients with localized CL, as well as the expansion of the use of oral therapies for CL caused by *L. mexicana* and *L. braziliensis*. For VL, the recommendation of liposomal amphotericin B was updated to strong, and the group of experts issued a **strong recommendation against** the use of miltefosine for patients with VL and the use of pentavalent antimonials for patients co-infected with LV-HIV⁸.

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TABLE 1 - Recommendations for the treatment of cutaneous, mucosal or mucocutaneous leishmaniasis in the Americas, according to recommendation strength and evidence.

Cutaneous leishmaniasis in adult patients		Cutaneous leishmaniasis in pediatric patients	
Recommendations	Recommendation Strength and Evidence	Recommendations	Recommendation Strength and Evidence
The application of intralesional pentavalent antimonials is recommended for patients with localized cutaneous leishmaniasis caused by <i>L. braziliensis</i> and <i>L. amazonensis</i> .	Strong recommendation, low certainty evidence	The use of miltefosine is recommended for pediatric patients diagnosed with cutaneous leishmaniasis caused by <i>L. panamensis</i> , <i>L. guyanensis</i> and <i>L. braziliensis</i> .	Strong recommendation, low certainty evidence
The use of miltefosine is recommended for adult patients diagnosed with cutaneous leishmaniasis caused by <i>L. panamensis</i> , <i>L. mexicana</i> , <i>L. guyanensis</i> and <i>L. braziliensis</i> .	Strong recommendation, low certainty evidence	The use of paromomycin is suggested for pediatric patients with cutaneous leishmaniasis caused by <i>L. panamensis</i> , <i>L. braziliensis</i> and <i>L. mexicana</i> .	Conditional recommendation, low certainty evidence
The use of pentamidine isetionate is suggested for patients diagnosed with cutaneous leishmaniasis caused by <i>L. guyanensis</i> .	Conditional recommendation, low certainty evidence	The use of pentavalent antimonials is suggested for pediatric patients diagnosed with cutaneous leishmaniasis when no other alternative is available.	Conditional recommendation, low certainty evidence
The use of thermotherapy is suggested for patients with localized cutaneous leishmaniasis caused by <i>L. braziliensis</i> , <i>L. panamensis</i> and <i>L. mexicana</i> .	Conditional recommendation, very low certainty evidence		
The use of paromomycin is suggested for patients with cutaneous leishmaniasis caused by <i>L. panamensis</i> , <i>L. braziliensis</i> and <i>L. mexicana</i> .	Conditional recommendation, very low certainty evidence		
The use of pentavalent antimonials is suggested for adult patients diagnosed with cutaneous leishmaniasis caused by <i>L. braziliensis</i> , <i>L. panamensis</i> , <i>L. amazonensis</i> , <i>L. peruviana</i> and <i>L. mexicana</i> .	Conditional recommendation, moderate to low certainty evidence		

Mucosal/mucocutaneous leishmaniasis	
Recommendations	Recommendation Strength and Evidence
The use of pentavalent antimonials with or without oral pentoxifylline is recommended for patients with mucosal or mucocutaneous leishmaniasis.	Strong recommendation, low to very low certainty evidence

Source: Guideline for the treatment of leishmaniasis in the Americas. Washington, D.C.; 2022. Pan American Health Organization. Licence: CC BY-NC-SA 3.0 IGO. <https://doi.org/10.37774/9789275125038>.

TABLE 2 - Recommendations for the treatment of visceral leishmaniasis in the Americas, according to recommendation strength and evidence.

Visceral leishmaniasis in immunocompetent patients		Visceral leishmaniasis in immunocompromised patients	
Recommendations	Recommendation Strength and Evidence	Recommendations	Recommendation Strength and Evidence
The use of liposomal amphotericin B is recommended for immunocompetent pediatric and adult patients with visceral leishmaniasis.	Strong recommendation, low certainty evidence	The use of liposomal amphotericin B is recommended for immunocompromised patients with visceral leishmaniasis.	Strong recommendation, very low certainty evidence
The administration of pentavalent antimonials or other formulations of amphotericin B is suggested for immunocompetent pediatric and adult patients with visceral leishmaniasis.	Conditional recommendation, low certainty evidence	The use of amphotericin B lipid complex or amphotericin B deoxycholate is recommended when liposomal amphotericin B is not available for immunocompromised patients with visceral leishmaniasis.	Strong recommendation, very low certainty evidence
The use of miltefosine is not recommended for pediatric and adult patients with visceral leishmaniasis.	Strong recommendation against, very low certainty evidence	The use of pentavalent antimonials is not recommended for immunosuppressed patients with visceral leishmaniasis.	Strong recommendation against, very low certainty evidence

Secondary prophylaxis of visceral leishmaniasis in immunocompromised patients	
Recommendations	Recommendation Strength and Evidence
For secondary prophylaxis in patients with visceral leishmaniasis and concomitant HIV infection, the administration of liposomal amphotericin B is recommended after the first episode of visceral leishmaniasis for all patients with a CD4 T cell count of less than 350 per mm ³ .	Strong recommendation, very low certainty evidence

Source: Guideline for the treatment of leishmaniasis in the Americas. Washington, D.C.; 2022. Pan American Health Organization. Licence: CC BY-NC-SA 3.0 IGO. <https://doi.org/10.37774/9789275125038>.





The Ministries of Health, through their respective technical areas and with the support of local experts, should evaluate the recommendations in the national context and determine the possibility of incorporating, guaranteeing and expanding patient access to treatment using safer and shorter-term therapeutic alternatives. Thus, it is important to consider the cultural aspects related to the acceptance of new therapeutic modalities, their availability in the region, costs and operating conditions to ensure efficient distribution and use.

In addition, as a strategy to disseminate these recommendations and to strengthen the specific knowledge of health professionals, the Pan American Health Organization (PAHO) will be

presenting a new version of the free Online Training Course on Leishmaniasis in the Americas: Diagnosis and Treatment – CL/ML and VL, at the Virtual Campus for Public Health.

Currently, PAHO's Strategic Fund (SF) incorporates all the drugs recommended for the treatment of leishmaniasis in the Americas, with the exception of paromomycin cream, which is not commercially available. To acquire the drugs, the SF carries out an annual planning with the countries, confirms engagement and consolidates the quantities needed to meet the national demands, which reduces the costs and guarantees the availability to meet regional needs. In the last two years, WHO has been coordinating global

discussions to secure subsidized prices and sufficient quantities from suppliers to serve endemic countries.

Despite the progress made by several countries in the adoption and availability of more than one option of medication, there is still much to be done to make other alternatives available and decentralized. There are also many challenges in the implementation of these recommendations, since national and subnational managers lack the financial resources needed for the acquisition and sustainability of the materials required for diagnosis and treatment. Other challenges include lack of production capacity or long production time, as well as delays in the distribution of medications, since most suppliers are exclusive. •



PROSPECTIVE STUDY OF LOCAL INTERVENTIONS FOR THE TREATMENT OF UNCOMPLICATED CUTANEOUS LEISHMANIASIS

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The use of intralesional antimonials (IL-AM) and thermotherapy (TT) for the treatment of cutaneous leishmaniasis (CL) are options that have been thoroughly explored, recommended by WHO, and indicated regionally by PAHO since 2013 as therapeutic alternatives in special situations. The new guideline for leishmaniasis treatment in the Americas, published by PAHO/WHO in 2022,¹ consolidates and expands the use of local interventions, bringing a strong recommendation for the treatment with IL-AM and conditional recommendation for TT for patients with localized CL with a maximum of three lesions of no more than 3 cm in diameter.

With efficacy similar to systemic antimonials, local therapies have advantages such as a better safety profile and easier use at the primary health care level, and in remote areas. In addition, they have shown to be cost effective. Some countries in the region have already included local treatments in their therapeutic guidelines, are revising the guidelines to include them, or have begun to use them in routine health care.

In this context, PAHO, in collaboration with the Ministries/Secretariats of Health of the respective countries, DNDi and Fiocruz-IOC, has proposed a prospective cohort study to assess the clinical response and tolerability of

treatment with TT or IL-AM in Mexico, Panama and Costa Rica.

The study will be conducted in some CL health care facilities in these countries, as identified by the respective Ministries of Health. Patients aged ≥ 12 years who meet the eligibility criteria defined in the protocol and have signed an informed consent will be included during a 12 month period.

Participants will be offered treatment with IL-AM (five applications with an average interval of five days) or TT (a single session) according to the protocol of each health care site, the local availability of the interventions, and the most favorable conditions for the participants. Follow-up will be conducted up to six months after the start of treatment, in accordance with the monitoring routine of each site. Clinical response will be evaluated three months after the start

of treatment based on the percentage of re-epithelialization/flattening of the lesions, according to the methodology described in Olliaro et al., 2013.²

The Laboratório de Pesquisas em Leishmaniose of the Oswaldo Cruz Institute will also identify the species of *Leishmania*, quantify the parasite load in the lesions before treatment and during the follow-up period (three and six months) if lesions continue active, and detect the *Leishmania* RNA Virus by PCR.

We expect, with this project, to contribute to a better management of patients affected by CL, expanding the adoption of local therapies for uncomplicated CL in the routine of health care services and helping reduce the morbimortality of this disease and the associated public-health costs in the respective countries. •



¹Guideline for the treatment of leishmaniasis in the Americas. Washington, D.C.; 2022. Pan American Health Organization. Licence: CC BY-NC-SA 3.0 IGO. <https://doi.org/10.37774/9789275125038>.

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LOCAL TREATMENTS FOR CUTANEOUS LEISHMANIASIS IN ETHIOPIA: FROM RESEARCH TO ACCESS

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While *L. tropica* and *L. major* predominantly cause cutaneous leishmaniasis (CL) in the Old World, a unique species is found in Ethiopia, *L. aethiopia*. The CL burden in Ethiopia is estimated at 20,000 to 40,000 new cases per year¹, disproportionately affecting children, adolescents and young adults. Over 30 million of Ethiopians live at risk for CL. The clinical manifestations of CL due to *L. aethiopia* are peculiarly pleiotropic; the localized form (LCL) is the most frequent manifestation, occurring in over 85% of cases², mostly presenting as single lesion on the face. Other forms include diffused cutaneous and mucosal forms. LCL in Ethiopia has a huge psychological and economic burden to the affected community, due to the predominant manifestation on aesthetically significant body parts in economically and socially active segments of the society and/or school-age children.

Local options including cryotherapy, intralesional injection of sodium stibogluconate (IL-SSG) and thermotherapy (TT) are recommended by the World Health Organization and have been widely used for the treatment of uncomplicated CL. In Ethiopia, IL-SSG administered weekly for up to six weeks is the standard treatment. Cryotherapy and TT are also included in the

Ethiopian national guideline. However, there is no robust evidence to support the recommendation of the proposed local interventions for *L. aethiopia*. Given the lack of local evidence, principally on the use of TT, the Armauer Hansen Research Institute (AHRI), in partnership with DNDi and the Instituto de Salud Carlos III (ISCIII), from Madrid, Spain, in coordination with the Ethiopian Ministry of Health's National Leishmaniasis Control Program, will conduct an integrated phase II/III, open label, multicenter, randomized clinical trial to determine the safety and efficacy of using TT in comparison to IL-SSG in uncomplicated LCL cases in four selected centers in Ethiopia. This study is part of the EDCTP RIA2020S funding "African Leishmaniasis: from Clinical Research to Access (LeishAccess Consortium; RIA2020S-3301)".

A total of 326 male and female participants aged ≥ 18 and ≤ 60 years old who meet the eligibility criteria defined in the protocol and from whom an informed consent form has been obtained will be enrolled in the trial. Participants will be randomized to receive:

- IL-SSG: 4 weekly IL injections at Days 1, 7, 14 and 21. Subjects who present less than 50% re-epithelialization or flattening of the lesion(s)

at day 28 will receive two additional weekly IL-SSG injections.

- Thermotherapy: one session, 50°C for 30-second applications.

Initial cure (at Day 90), late responders (Day 105) and final cure (at Day 180) will be assessed based on the percentage of re-epithelialization of the ulcer(s) (for ulcerated lesions) or flattening and/or no signs of induration of the lesion(s) (for non-ulcerated lesions) as compared to Day 1.

As secondary objectives, this study will also conduct a molecular characterization and phylogenetic analysis in *Leishmania* isolates, which will be performed by ISCIII, as well as evaluate quality of life and CL scars based, respectively, on the Dermatology Life Quality Index questionnaire and the Vancouver Scar Scale, coupled with the Patient and Observer Scar Assessment Scale. The ultimate goal is to provide evidence to the MoH about the safety and efficacy of TT for the treatment of CL due to *L. aethiopia* and, if results are positive, to promote its adoption on a routine basis.

The study has already been approved by AHRI, ISCIII and National Ethics Review Committees, and recruitment is expected to begin by Q3 2022. •

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FIRST-IN-HUMAN STUDY TESTING CPG ODN D35, A TOLL-LIKE RECEPTOR 9 AGONIST, PROVES ITS SAFETY AND IMMUNE EFFECT IN HEALTHY VOLUNTEERS

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Toll-like receptors (TLR), which recognize conserved microbial determinants, activate the cells of the innate immune system to limit the early spread of pathogens while promoting the development of antigen-specific immunity. CpG ODN D35 is a class A CpG ODN TLR9 agonist, specifically developed to enhance the treatment of cutaneous leishmaniasis (CL). CpG ODN D35 is a short, single-stranded synthetic DNA molecule that contains un-methylated Cytosine-Guanine (CG) dinucleotide motifs that can be detected by TLR9 on plasmacytoid dendritic cells (pDCs) in humans. In animal models, it stimulates maturation and activation of pDCs and production of proinflammatory cytokines, such as *IFN- α* and *IFN- γ* , which are required for control of the *Leishmania* infection. CpG ODN D35 has little or no effect on B cells and does not

foster the Th2 type responses associated with other classes of CpG ODN.

A First-In-Human Phase 1 clinical study investigated the safety, pharmacokinetics, and immune effects of a single ascending dose of CpG ODN D35 administered subcutaneously in healthy male subjects aged between 18 and 50 years. The study consisted of 3 cohorts (7.5, 22.5 and 67.6 mg) of 8 male subjects randomly assigned to receive a single dose of CpG ODN D35 (6 subjects) or placebo (2 subjects).

Overall, CpG ODN D35 was found to be well tolerated and safe, with a limited number of injection site reactions, mainly redness and tenderness, ranked as mild or moderate and all self-limited. A consistent pattern of haematological changes (WBC decrease at days 4-7, slight neutrophil

and moderate basophils decrease at days 4-7, lymphocyte decrease on day 2, slight monocyte increase on day 4) was observed in a dose-dependent manner – all transient and non-clinically significant. A dose-dependent increase of CXCL10 concentrations in plasma was observed with no increases in TNF α or IL-6 values.

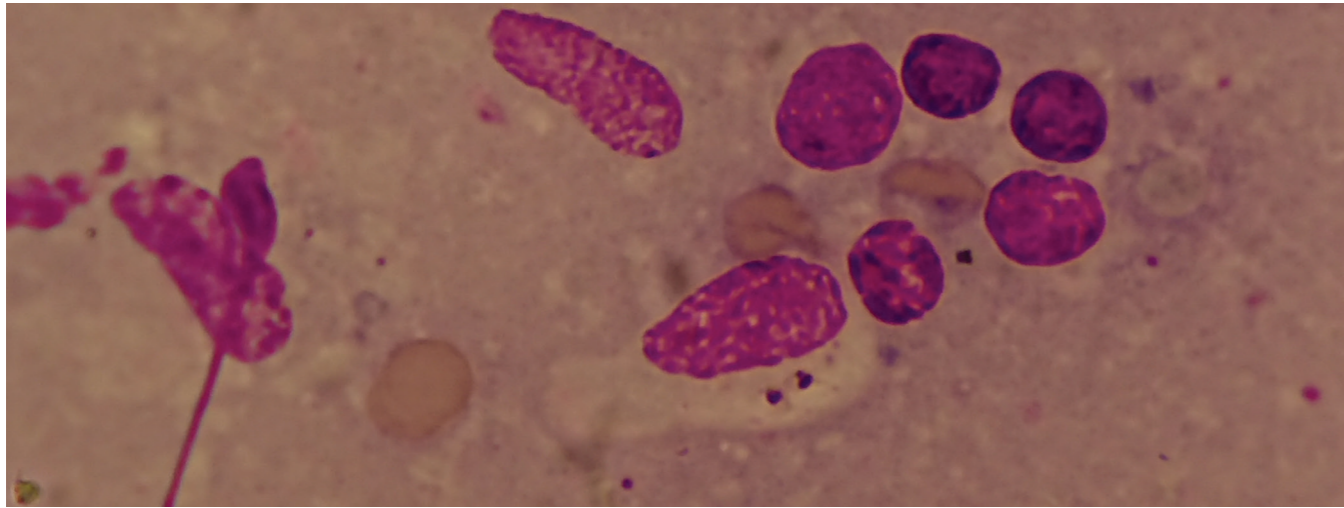
Given the encouraging results of this first study in healthy volunteers, a multiple ascending dose study is being planned in NW patients with CL lesions. The study will be a Phase 1b, single centre, randomised, open label study, evaluating multiple ascending doses of CpG ODN D35 administered subcutaneously in male and female subjects aged between 18 and 50 years with uncomplicated cutaneous leishmaniasis. Current plans are to initiate by Q4/2022. •





CURRENT OVERVIEW AND PERSPECTIVES IN THE DEVELOPMENT OF DIAGNOSTIC TESTS FOR CUTANEOUS LEISHMANIASIS

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Laboratory diagnosis is still one of the major challenges for the control of cutaneous and mucocutaneous leishmaniasis, diseases that are still present in tropical countries on many continents, with a broad range of clinical manifestations and potentially toxic treatment. Parasitological methods based on the direct observation of the amastigotes or the culture of promastigotes, although highly specific, have limited sensitivity. On the other hand, traditional molecular methods are highly accurate, but also complex and costly, characteristics that limit their use. Another limiting factor in diagnostics is the use of invasive methods for sample collection, such as a biopsy or the subcutaneous aspiration of the lesion, both of which must be performed by specialized professionals and may cause bleeding, infection and pain to the patients. Due to these challenges, the development of high-performance techniques that are simple to perform from samples collected in a minimally invasive way is a priority strategy for both the cutaneous and mucocutaneous forms of leishmaniasis, to ensure access to diagnostics, decrease time until treat-

ment, and minimize risks and morbidity for patients. To guide the development of diagnostic strategies that could be employed at all levels of health systems, the World Health Organization (WHO) has proposed a set of minimum parameters for the ideal test, focusing on accessibility, high performance, simplicity of use and rapidity of results (Mabey *et al.*, 2004). Additional criteria regarding real-time connectivity and easy collection of samples were added later, creating the acronym REASSURED (Land *et al.*, 2019). In addition, the Foundation for Innovative New Diagnostics (FIND) has recently proposed, based on expert opinion, a product target profile for a point-of-care diagnostic test for cutaneous leishmaniasis, that should present a minimum of 95% sensitivity and 90% specificity (Cruz *et al.*, 2019).

The search for a test that fulfills the minimum requirements may leverage significant changes in the current approaches to the disease, but will also require substantial investment, both material and human, for the identification and validation of new diagnostic tools. Given the current evidence, methods based on

the isothermal amplification of DNA or other rapid immunological methods associated with minimally invasive sample collection approaches have become the most promising strategies in the quest for the ideal diagnostic. Collecting clinical samples in a minimally invasive way, whether using swab, cytology brush or paper filter, has become a viable alternative to lesion biopsy. In fact, there is evidence that the *Leishmania* concentration may be higher when obtained through these techniques, as compared to the conventional invasive techniques (Suárez *et al.*, 2015; Sevilha-Santos *et al.*, 2019). This may be explained by the higher parasitic load in the upper layers of the skin and by the higher representation of the sample due to the larger extension of the lesion covered by the collection. Among the advantages of minimally invasive techniques are the feasibility of performing diagnostics in remote areas with minimum infrastructure, by staff with a secondary level of education with some training, the lower risk of complications for the patients, and a potentially lower cost. Minimally invasive approaches, when associated to molecular techniques

centered on specific targets for the diagnosis of leishmaniasis, have achieved satisfactory performance (Daoui *et al.*, 2020). Using polymerase chain reaction (PCR) with a kDNA target, samples obtained by swabbing and biopsy present similar sensitivity for the diagnosis of patients with both the cutaneous and the mucocutaneous forms of the disease, close to 93% (Faria *et al.*, 2022; Boni *et al.*, 2017; Gomes *et al.*, 2017).

Among the isothermal amplification techniques, the most commonly used is LAMP (Loop-mediated Isothermal Amplification). Its advantages go beyond its high specificity, which is ensured by the set of primers that are used, being also related to its accessibility and ease of use and reading by visualizing the byproducts of the reaction. Data gathered in a recent systematic literature review revealed sensitivity ranging from 80% to 99%, and specificity ranging from 91% to 98% (Erber *et al.*, 2022). Even though molecular isothermal amplification methods do not fulfill

all the requirements of a true point-of-care diagnostic test due to the DNA extraction step, they are easy to use and require limited infrastructure, being feasible to implement even in contexts without any resources. Multicentric studies assessing how LAMP performs in real-world conditions and with different targets should be encouraged for further standardization.

Immunology techniques in different platforms connected to quick reading systems are also important candidates for a point-of-care test, especially those based on the detection of species-specific antigens, which have shown promising preliminary results (Freire *et al.*, 2021). One of the few commercial tests available for the diagnosis of the cutaneous form of the disease is CL Detect™ (InBios International Inc., Seattle, WA, USA), a rapid immunochromatography test that detects *Leishmania* antigen from samples obtained by means of biopsy or cytological brush of the ulcerated lesion with up to four months

of evolution. Validation studies have shown a performance of more than 60% in regions where *L. major* or *L. tropica* predominate (Bennis *et al.*, 2018; Vink *et al.*, 2018). However, in other endemic regions, a significantly lower sensitivity, of approximately 30%, has been reported (De Silva *et al.*, 2017; Schallig *et al.*, 2019; van Henten *et al.*, 2022).

In summary, despite promising preliminary results and some performance requirements already agreed upon, it is undeniable that there is long road ahead in the quest for the ideal test, especially considering the global reach of the disease and the wide diversity of its species and manifestations. The development and validation of new diagnostic tools guided by the objectives now described must become a priority for the current research on leishmaniasis. The adherence of both the scientific community and the research funding agencies to a common strategic agenda is paramount for the real transformation of the diagnostics scenario for dermic leishmaniasis. •

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PEDIATRIC TREATMENT: AN UNMET NEED

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Tegumentary leishmaniasis (TL) is a vector-borne disease caused by parasites of the genus *Leishmania*. This disease has a pleomorphic clinical behavior that varies from self-contained forms to chronic and severe manifestations which can be both cutaneous and mucosal. The complexity of TL in humans depends on (i) the diversity of the immune response, which is influenced by genetic and acquired factors, (ii) the infecting *Leishmania* species (more than 18 species affect humans), which can stimulate or evade host immunity, and (iii) environmental conditions that can affect human exposure to the pathogen, but also modulate its immune response.

This spectrum of clinical manifestations is also affected by other factors, such as congenital or acquired immunosuppressive conditions, the age at which infection occurs, or the coexistence of transmissible and non-communicable comorbidities. Age is known

to influence multiple aspects, including clinical manifestations, the therapeutic response, and the risk of progression to mucocutaneous leishmaniasis (ML). Several infections show a greater propensity to severity in infants or preschool children. This would be due to the immaturity of the innate and adaptive immune system, which acquires memory and strengthens with age and subsequently declines in old age. Immunological senescence, which affects the levels of IL-2, IFN- γ , T and B cell counts, and the excessive accumulation of regulatory T cells could be extrapolated to understand the immunological mechanisms occurring at the other age range; however, the lack of verification of these assumptions demonstrates the limited knowledge of the pathobiology of TL in the pediatric population.

The therapeutic failure of pentavalent antimonials (Sb⁵⁺) in the pediatric population varies between 40 and 70%, with

the age group < 5 years having three times more risk of therapeutic failure. Miltefosine has shown to be an effective alternative for children, in spite of significant accessibility limitations due to the high cost and the absence of pediatric formulations. Amphotericin deoxycholate and other formulations are essentially used as a second-line therapy, with the additional limitation that long periods of hospitalization are required for safe administration, although the adverse effects are less severe and frequent than those reported in adults (50 to 70% less). It has been shown that the difference in the therapeutic response between adults and children could be due to a lower bioavailability of Sb⁵⁺ and miltefosine as a result of the accelerated pharmacological metabolism in the pediatric population. This insufficient exposure could be reversed using allometric schemes (according to weight and height, as well as nutritional status) of drug administration.

Considering that more than 50% of those affected by TL in endemic areas are children, it is necessary to prioritize various aspects of the diagnosis and management of the pediatric population. As could be seen in the therapeutic aspects, clinical trials in this age group are scarce for several reasons: (i) children are a protected group in clinical trials and there is an excess of zeal in ethical regulations, which paradoxically limits the exploration of diagnostic and therapeutic alternatives in this age group; (ii) the lack of support from the pharmaceutical industry or funding institutions for leishmaniasis research; (iii) the neglected nature of leishmaniasis that confines its endemicity to low-income populations with poor access to health care. •



PAROMOMYCIN GEL FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS: THE CHALLENGES BEHIND THE DEVELOPMENT OF A PHARMACEUTICAL PRODUCT BY BRAZILIAN PUBLIC INSTITUTIONS

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The urgent need for a safe, comfortable and effective treatment for the approximately 40,000 people who develop cutaneous leishmaniasis every year in the Americas (PAHO, 2021) has driven the efforts undertaken in the development of paromomycin gel. Despite the good results obtained in a Phase III clinical trial of a paromomycin cream formulation developed by the Walter Reed Army Medical Center in the United States (Sosa *et al.*, 2019) for topical treatment of cutaneous leishmaniasis, this product is not yet available to the people affected by this disease in the Americas.

The development of the paromomycin sulfate gel formulation began in the

Pharmacy School of the Universidade Federal de Minas Gerais (UFMG) in 2000. In light of the results obtained in experimental studies (Gonçalves *et al.*, 2005), Fiocruz and UFMG started negotiating a technology-transfer agreement that would allow Farmanguinhos/Fiocruz to industrially produce the formulation together with the technology-management units CTIT/UFMG, GESTEC and Programa de Desenvolvimento Tecnológico em Insumos para a Saúde – PDTIS/Fiocruz. The first challenge encountered in the development journey was the harmonization of the legal understanding of the agreement terms by UFMG's and Fiocruz' Federal Legal Bureaus, particularly in regards to

its commercialization clause. After six years of negotiations, the solution found was to postpone the inclusion of this topic until a potential partnership with the private sector is under review.

The second big challenge was technical. The paromomycin gel formulation is hydrophilic, a characteristic that makes it easier for the skin to absorb, but also less stable in physicochemical terms. For this reason, the R&D team at Farmanguinhos/Fiocruz had to develop eight new formulations, which were all evaluated in the IRR/Fiocruz's animal models. In addition, it was necessary to develop and validate analytical methodology to support stability studies of each proposed formulation. This effort took two years and involved investments, research and errors and successes.

At the same time, *in vivo* biological studies were conducted to evaluate alternative formulations and better combinations of the topical paromomycin gel formulation and the least aggressive systemic treatment available. Following stability and pre-clinical safety and efficacy criteria, the extemporaneous formulation of 10% paromomycin gel was chosen as the test product. This means that the solid constituent parts of the paromomycin gel are packaged in sachets and the gel must be reconstituted with water when dispensed to the patient.

The biological activity analysis of an experimental batch of paromomycin gel in hamsters with *Leishmania Viania brasiliensis* showed higher clinical efficacy when applied twice a day for 30 days, in combination with a single





intralesional application of meglumine antimoniate on the first day of treatment. The combination reduced the size of the lesions by 100%, a significantly higher percentage than the one observed in the groups treated with monotherapy (Alves L. L., 2018).

The third big challenge arose from the changes in the presentation of the product. The Farmanguinhos facilities are certified by the Agência Nacional de Vigilância Sanitária - ANVISA for the production of semi-solids, but not for the production of the extemporaneous gel. After an extensive search for pharmaceutical companies that could produce the pilot batches, the pharmaceutical laboratory Eurofarma showed interest in partnering with Fiocruz. The legal requirements of the R&D process required that Eurofarma respond to a Public Call for Proposals and also to a Public Notice issued by Fiocruz's Fundação de Apoio a Pesquisa - Fiotec, which is managing the funding provided by the Banco Nacional de Desenvolvimento Econômico e Social - BNDES and the Inova Program of Fiocruz. As of this moment, the service agreement has yet to be signed, at which point Farmanguinhos will coordinate the pilot batch production procedures and the fulfilment of the regulatory requirements.

The fourth big challenge is the funding. We should highlight the importance of having strong public institutions working independently and in alignment with each other. This product is funded by public investment. In 2016, part of the costs of the formulation's development and funding for the

conduction of the clinical trial were ensured through a long selection process by the BNDES' FUNTEC Program, with the positive advice of the Ministry of Health (Grant Agreement for Non-reimbursable Financial Collaboration No. 15.2.0473.1). This type of contract requires that at least 10% of the amount be provided as financial counterpart by the beneficiary, in this case Farmanguinhos, which suffered severe financial constraints in the following years. The Fiocruz Presidency provided this contractual funding requirement using its own resources and those of Inova Program. Farmanguinhos and IRR/Fiocruz are regularly investing in the project by providing materials, equipment and personnel.

The fifth big challenge is the availability of the Active Pharmaceutical Ingredient (API). Despite our best efforts, we could identify only one supplier of paromomycin certified in good manufacturing practices: the Olon company, from Italy. After all these years of project development and the rise in the Euro/BRL exchange rate in 2020/2021, the API costs doubled. Therefore additional funding, and a few months' extension of the project, were necessary, also because of production delays caused by the COVID-19 pandemic.

After the type of formulation was chosen, the clinical teams from IRR/Fiocruz and the Plataforma de Pesquisa Clínica/Fiocruz set up the Phase III, multicentric, open-label, randomized, controlled trial to assess the non-inferiority of efficacy and safety of the extemporaneous paromomycin gel formulation for topical use in adult and ad-

olescent patients with cutaneous leishmaniasis. Arm 1: 10% paromomycin gel, topical use, twice a day for 30 days, combined with a single intralesional application of meglumine antimoniate on the first day of treatment; Arm 2: three intralesional applications of meglumine antimoniate 15 days apart (the treatment recommended by the Ministry of Health). The trial will be conducted in three research centers: IRR/Fiocruz, Hospital Presidente Vargas - São Luís do Maranhão, and Hospital Universitário Clemente de Faria - Universidade Estadual de Montes Claros (Unimontes). The project was approved by CONEP - Comissão Nacional de Ética em Pesquisa do Conselho Nacional de Saúde under the number 35331720.8.0000.5091 and registered on the Plataforma de Registro Brasileiro de Ensaios Clínicos - REBEC under the number RBR-4ypn69.

In December of 2020, Farmanguinhos submitted electronically the Clinical Drug Development Dossier (Dossiê de Desenvolvimento Clínico de Medicamento - DDCM) to ANVISA. Two requests for clarifications and additional information made by the Agency were answered on time. The Agency has not yet given its final answer. The complexity of the forms and ANVISA's response times represent the sixth big challenge.

The development trajectory of paromomycin gel for the topical treatment of cutaneous leishmaniasis was undergirded by national public institutions that represent the strengths and weaknesses of a country in search of autonomy and sovereignty for its Health Industrial Complex. •

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BUILDING A PATHWAY FOR TRANSLATIONAL DRUG DEVELOPMENT OF ORAL TREATMENTS FOR CUTANEOUS LEISHMANIASIS



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Cutaneous leishmaniasis (CL) remains a neglected disease for which new, safe, and orally efficacious drugs are urgently required. DNDi has been actively engaged in leishmaniasis R&D for over 15 years, and, together with multiple partners, has built an unprecedented portfolio of late-lead series and preclinical and clinical candidates originating from different chemical classes with different mechanisms of action against *Leishmania* parasites.

This promising portfolio of new chemical entities (NCEs) is currently being developed for visceral leishmaniasis (VL), aiming at the identification of short-course monotherapy(ies) and/or combination treatments. DNDi's CL programme strategy is to leverage this rich portfolio by expanding its development to simultaneously identify new oral CL treatments and potential combinations with immunomodulators. This objective is strongly supported by previously generated data and ongoing investigative efforts, which clearly demonstrate the capacity of these candidates to be "expanded" for CL. Data includes potent *in vitro* profiles against a panel of CL-causing strains, *in vivo* proof-of-concept studies in animal models, and skin distribution potential obtained for some of these NCE candidates^{1,2}.

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However, translational CL research is notoriously challenging, and there are multiple gaps precluding the upstream clinical development of drug candidates for this disease, including (i) the need for a better understanding and prediction of how orally administered drugs distribute into the skin and interact with *Leishmania* parasites in the tissue, and (ii) the lack of robust and standardized animal models mimicking skin infection with some of the most relevant *Leishmania* species — such as *L. braziliensis*.

To address these important questions and build an innovative pathway for translational drug development of oral NCEs for CL, DNDi has partnered with an international group of academic experts, public-private partnerships, and industry from Brazil, the United Kingdom, the Netherlands, and France, to combine expertise in CL, drug discovery and development, pharmacokinetics (PK) and skin drug delivery, basic parasite biology, quantitative pharmacology, and modelling (see figure for project partners and responsibilities). This multidisciplinary project has recently been granted funding from Dioraphte, a private foundation from the Netherlands

(<https://www.dioraphte.nl/en/>), for a three-year research program. The project is currently in the preparation phase.

The overall activities of the project are divided into two work packages that are connected to provide a rational workflow. The first work package comprises detailed analysis of skin and plasma exposure-efficacy response of promising antileishmanial NCEs using CL mouse models. Multiple technological platforms (microdialysis, liquid chromatography mass spectrometry, and quantitative mass spectrometry imaging) will be used for PK studies and bioanalysis, generating quantitative and spatial information about drug distribution in the different layers of healthy and infected mouse skin. State-of-the-art pharmacokinetic-pharmacodynamic (PKPD) modelling techniques will be employed to establish exposure-response relationships and determine required PK targets for these NCEs. Modern physiologically based pharmacokinetic (PBPK) modelling will be used for interspecies translation and scaling, providing fundamental insights on how systemically administered drugs distribute into skin tissues and interact with *Leishmania* parasites. By applying

this new knowledge, improved predictions of human efficacious dose(s) will be generated, and measurable and testable human PK targets will be defined for future use and validation during CL Phase II clinical trials with these NCEs.

The second work package comprises the development of new tools for CL drug discovery, namely a long-awaited bioluminescent mouse skin model using *L. braziliensis*, the most clinically relevant and difficult to treat causative agent for CL in the Americas.

Building this CL drug development pathway will allow the generation of preclinical knowledge with high translational value for promising oral NCEs and will increase the probability of successful advancement of such candidates into future clinical trials.

As a result of this project, promising NCEs will be progressed faster to patients, alleviating unnecessary suffering in affected populations in endemic countries. In addition, the application of this translational platform could possibly be expanded to post-kala-azar dermal leishmaniasis, mucocutaneous leishmaniasis, and other parasitic skin diseases. •

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