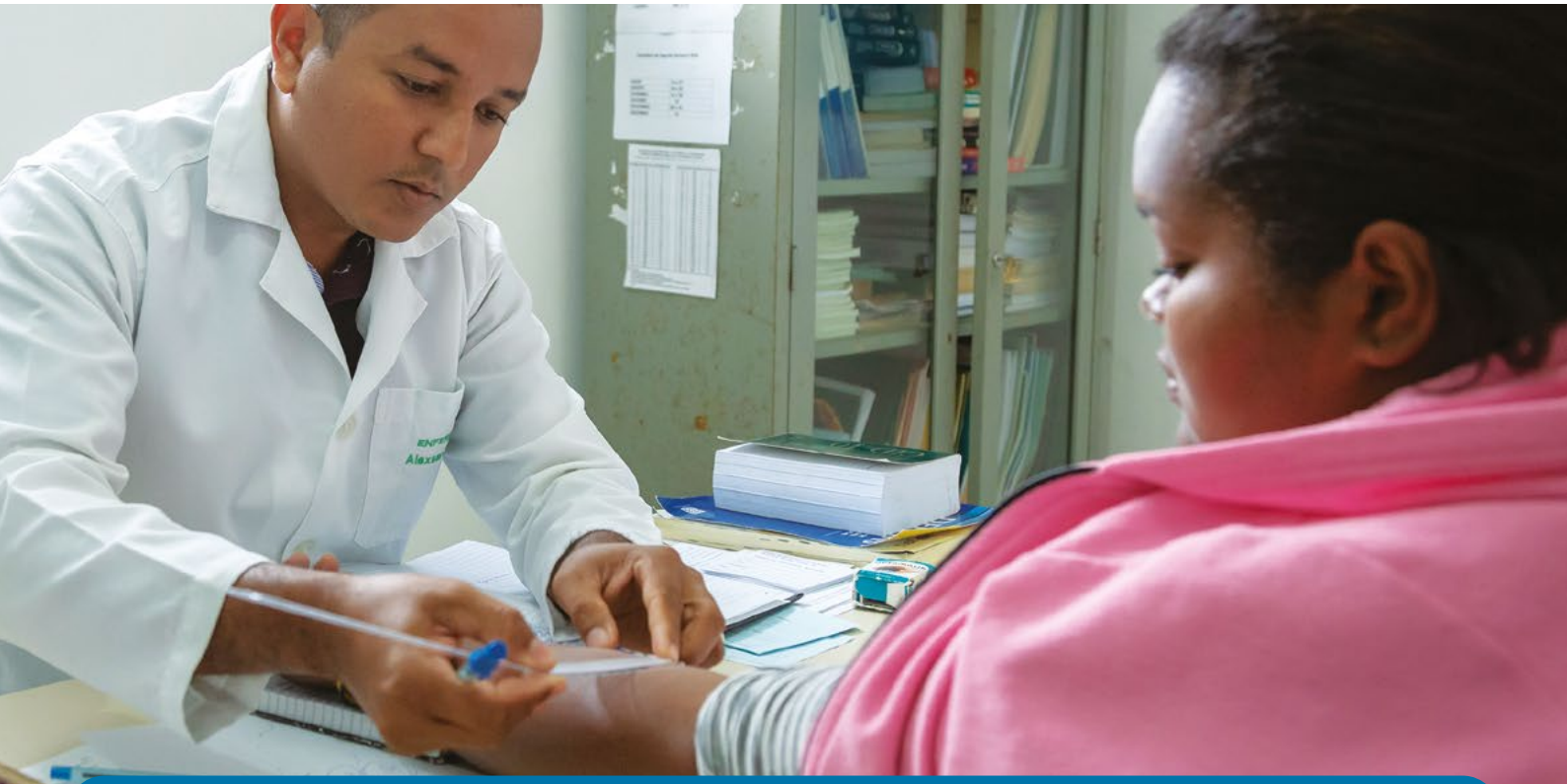
InfoLEISH

redeLEISH newsletter - 8th edition

May 2024



EDITORIAL - BYRON ARANA, DNDi

Twelve years ago, the DNDi leishmaniasis team embarked on a mission with passion, commitment, and hope to find safer and more effective treatments for cutaneous leishmaniasis (CL). As part of this effort and with the goal of fostering the exchange of information, knowledge, and developing collaborative activities, redeLEISH was established ten years ago. As we celebrate redeLEISH's tenth anniversary, we want to express our gratitude for the support, enthusiasm, and dedication of all its members and collaborators who directly or indirectly have contributed to achieving redeLEISH's objectives.

Developing new treatments for CL has indeed been a challenging journey, fraught with setbacks. A major challenge is the lack of funding attributed, among other factors, to the fact that the disease is not considered fatal, disregarding other important problems caused by the disease, like stigma, skin scars, segregation, mental health, etc., which affect more than a million of people living with CL every year.

Despite these difficulties, progress has been made. For instance, existing interventions such as thermotherapy have now become available in most Latin American countries, an effort that has been led by the Pan American Health Organization with the support from the Drugs for Neglected Diseases Initiative.

Our attempts to improve the efficacy of current treatment interventions have led us to assess the combination of a single application of thermotherapy plus oral miltefosine for three weeks. Final results of a Phase III study conducted in 6 institutions participating in redeLEISH in Brazil, Bolivia, Panama, and Peru will be known by the end of 2024.

An exciting development in the field of immune host therapies is the transition of CpG D35 from preclinical to early clinical development. In healthy volunteers, the administration of CpG D35 showed to be safe and able to promote the immune response which has been associated with the control of the disease. We have been advancing with a multiple ascending dose study in CL patients.

Looking ahead, for the last year the team has been working towards initiating in 2025 a Phase II study to test the safety and efficacy of a new oral compound developed by Novartis, which in animal and *in vitro* studies has shown to be active against *Leishmania* species causing CL.

All together it has been an exciting journey, with many reasons to not lose hope and continue supporting coordinated efforts in the search for new treatments for cutaneous leishmaniasis. •

Summary

IDENTIFICATION OF NOVEL GLYCOGEN SYNTHASE KINASE 3 (GSK3) INHIBITORS AS POTENTIAL ANTI-LEISHMANIA THERAPY

PRISCILA Z. RAMOS, **KATLIN B. MASSIRER**, **GABRIELA BARREIRO** and **CAROLINA M. C. CATTA-PRETA**, Centro de Química Medicinal (CQMED), Center for Molecular Biology and Genetic Engineering (CBMEG), UNICAMP, Brazil, **TANIRIS C. BRAGA**, Centro de Química Medicinal (CQMED), Center for Molecular Biology and Genetic Engineering (CBMEG), UNICAMP, Brazil/Eurofarma Laboratórios S/A, Brasil, **JULIANA I. AOKI,** Eurofarma Laboratórios S/A, Brasil/Institute of Biology, UNICAMP, Brazil, **JÚLIA L. MONTEIRO**, **VITOR M. ALMEIDA** and **CARINE C. DREWES,** Eurofarma Laboratórios S/A, Brazil, **ADRIANO C. COELHO**, Institute of Biology, UNICAMP, Brazil, **JADEL M. KRATZ** and **LUIZA CRUZ**, Drugs for Neglected Diseases Initiative Latin America, Lead Optimization Latin America, Brazil, **RONALDO A. PILLI**, Institute of Chemistry, UNICAMP, Brazil, **RAFAEL M. COUÑAGO**, Eurofarma Laboratórios S/A, Brazil/Structural Genomics Consortium, UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.

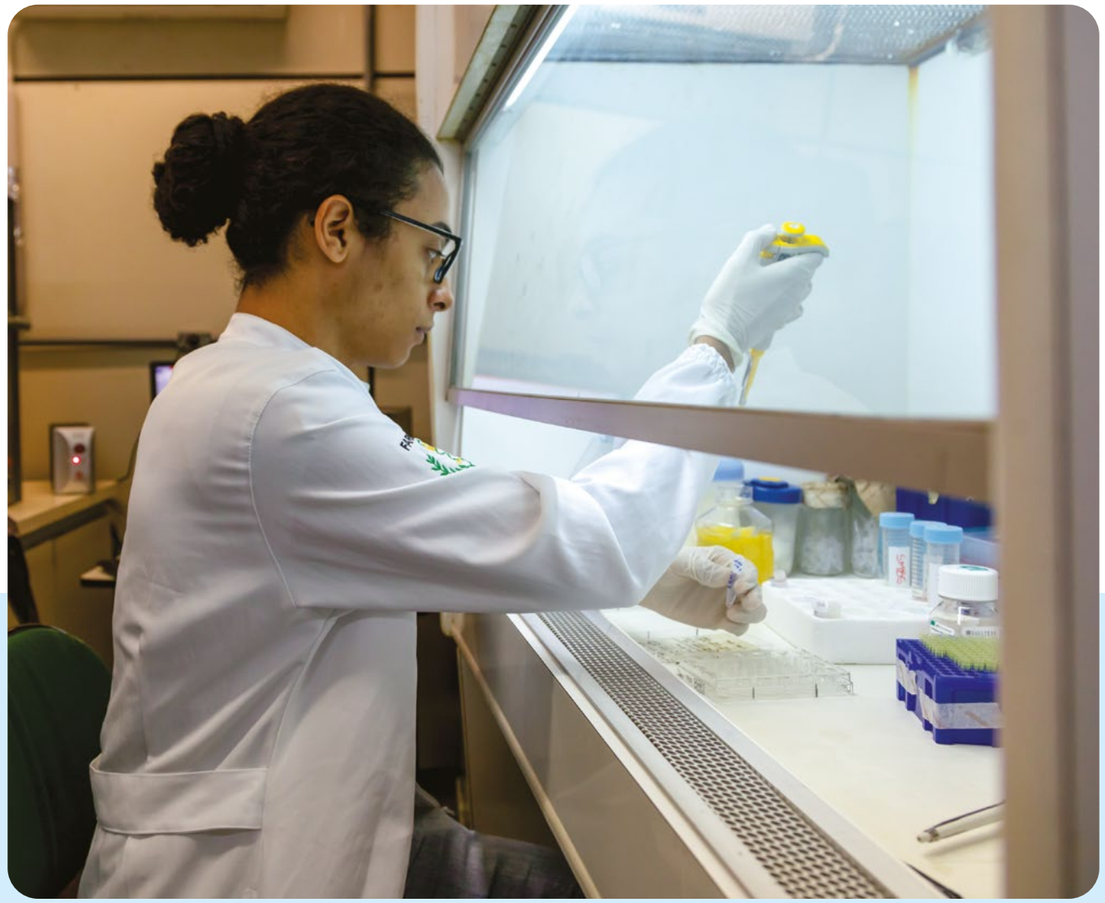
Over the past decades, much progress has been made in the treatment of leishmaniasis, leading to improved outcomes and a better understanding of the disease. Nevertheless, current treatments are still hampered by issues such as toxicity, high costs, emerging drug resistance, lengthy treatment durations, and limited efficacy.

These challenges underscore the need for continued research on target-based drug discovery strategies. Such strategies focus on identifying specific biological pathways or proteins crucial for the parasite's survival and virulence, offering the potential for developing more effective, less toxic therapeutic approaches.

To explore the use of target-based strategies and identify potential therapeutic targets for leishmaniasis, we established a multidisciplinary consortium with researchers from the University of Campinas (UNICAMP), the Center of Medicinal Chemistry at UNICAMP (CQMED), DNDi and Eurofarma. This initiative, named Leish-SBDD, leverages the expertise of over 20 researchers and receives support from both public and private funding.

While target-based approaches have been successful in various clinical areas, they have not yet yielded medications for leishmaniasis or other diseases caused by eukaryotic parasites, such as Chagas disease and malaria. A significant challenge in applying target-based strategies to parasitic protozoa arises from a general lack of knowledge about the complex biology of these organisms. This knowledge gap is partly due to the limited availability of molecular and chemical tools required to identify proteins or biological processes crucial for parasite survival within the human host. These essential proteins, once identified, would serve as ideal targets for innovative therapies.

The recent implementation of CRISPR-Cas9 gene knockout technology in *Leishmania* has enabled researchers to identify genes which are essential for parasite differentiation and survival. This has led to the identification of several *Leishmania* genes encoding protein kinases that play a crucial role in the parasite's survival within a mammalian host[1]. Protein kinases serve as master regulators of cell signaling cascades, controlling processes such as cell proliferation and death. Consequently, they have been extensively explored as therapeutic targets for various human diseases. This discovery has paved the way for leveraging the extensive medicinal chemistry toolkit available for human protein kinases to *Leishmania* drug discovery. »

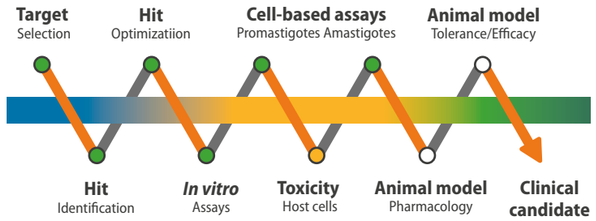


Reference

1. Baker, N., Catta-Preta, C.M.C, Neish, R. *et al.* Systematic functional analysis of *Leishmania* protein kinases identifies regulators of differentiation or survival. *Nature Communications*, v. 12, n. 1, p. 1244, 2021.

SBDD-Leish

Structure-Based Drug Discovery Pipeline



Screening Cascade:

1,400 kinase innhbitors

enzyme assays

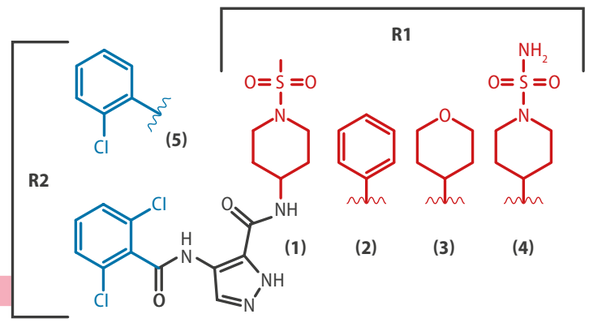
Prioritized compounds

cell-based assays
chemical properties

Compound 1

iterative, structure-based DMTA cycles

Optimized compounds 2-5



Compound activity:

	In vitro purified enzyme (Ki - μM)*			Cell-based assays (EC_{50} - μM)*		
	*Linf*GSK3α	*Linf*GSK3β	Free-living Promastigotes	Toxicity - Mouse BMDM	Mouse BMDM - Internalized Amastigotes	Selectivity Index (SI)*
1	3×10^{-5}	2×10^{-5}	3×10^{-1}	8	1	8
2	>1	2×10^{-4}	3	52	3	17
3	5×10^{-1}	2×10^{-5}	5×10^{-2}	12	2	6
4	2×10^{-5}	2×10^{-5}	5×10^{-1}	17	1	17
5	1×10^{-5}	5×10^{-5}	9×10^{-2}	19	1	19
Miltefosine	Not tested	Not tested	17	76	2	38

* K_i - inhibitory constant; EC_{50} - Half maximal effective concentration; SI - Selectivity Index is calculated by dividing the EC_{50} values of a compound obtained in host macrophages by those obtained for macrophage-internalized amastigotes.

Figure 1 – The SBDD-Leish Consortium's Target-based Drug Discovery Pipeline identified promising GSK3 inhibitors exhibiting anti-leishmanial activity comparable to miltefosine, an approved drug for visceral leishmaniasis.

Our SBDD-Leish Consortium chose to investigate the therapeutic potential of two kinases identified as essential in genetic studies: glycogen synthase kinase 3 alpha and beta from *Leishmania infantum* (*Linf*GSK3α and *Linf*GSK3β), the causative agent of visceral leishmaniasis in Latin America. Additional evidence supporting *Linf*GSK3β as a promising therapeutic target was provided by Xingi and colleagues, who demonstrated that inhibitors of this enzyme were active against the parasite in culture[2]. One challenge when targeting parasite kinases with small molecule inhibitors is the potential for these compounds to also inhibit human host kinases, resulting in toxic effects. Therefore, a major objective of our project was to develop *L. infantum* GSK3 inhibitors that were effective against the parasite while remaining harmless to human cells. We used miltefosine, a drug clinically approved for treating visceral leishmaniasis, as a benchmark to evaluate the anti-*Leishmania* activity and host cell toxicity of our novel compounds.

To achieve this goal, our Consortium developed the necessary reagents and assays to support a target-based drug discovery pipeline (Figure 1). This involved producing purified recombinant *Linf*GSK3α and *Linf*GSK3β to develop *in vitro* assays and measure the activity of potential inhibitors of these enzymes. Additionally, we determined the crystal structure of *Linf*GSK3β bound to kinase inhibitors through x-ray crystallography to gain a detailed understanding of the interaction between the enzyme and its inhibitors. We also established cell-based assays to assess the impact of compounds on the viability of *L. infantum*. This was done both in promastigotes and in clinically-relevant intracellular amastigotes – using bone-marrow derived mouse macrophages (BMDM) as host cells. Finally, we curated a collection of 1,400 kinase inhibitors anticipated to exhibit activity against *Linf*GSK3α and *Linf*GSK3β.

Indeed, screening our kinase-focused collection identified several potent inhibitors of *Linf*GSK3α and *Linf*GSK3β. These inhibitors were further evaluated in cell-based assays using *L. infantum* promastigotes and intracellular amastigotes. Results from enzymatic and phenotypic assays, along with an assessment of the physicochemical properties of the active compounds, guided our selection of pyrazolcarboxyamide compound **1** as the starting point for a medicinal chemistry program aimed at enhancing its potency against *Linf*GSK3α and *Linf*GSK3β, as well as its anti-leishmanial activity against macrophage-internalized *L. infantum* amastigotes, while simultaneously reducing its toxicity towards mammalian host cells.

Our medicinal chemists employed the crystal structure of *Linf*GSK3β to gain a deeper understanding of its interaction with pyrazolcarboxyamide compounds. They then designed modifications to compound **1** with the aim of enhancing its activity and pharmacological properties. This initiated iterative Design-Make-Test-Analyze cycles, encompassing the *in vitro* testing of 35 novel molecules against the *Leishmania* kinases, as well as the parasite and mammalian host cells in culture.

These efforts resulted in the discovery of optimized compounds (**2**-**5**) that were more potent against the purified enzymes and, more importantly, up to two-fold more active in intracellular *L. infantum* amastigotes than miltefosine. These compounds, however, were more toxic to mammalian host cells than miltefosine, as shown by their Selective Index (SI).

While our progress has shown promise, further work lies ahead. Our next steps will focus on the continued assessment and improvement of the pharmacological properties of our compounds, as well as gaining a better understanding of their potential toxicity toward mammalian host cells. •

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KINETOPLASTID PROTEASOME INHIBITION, A NOVEL TREATMENT OPPORTUNITY FOR THE MANAGEMENT OF LEISHMANIASIS

GERHILD ANGYALOSI, **NATASHA HOCHBERG** and **SRINIVASA P S RAO**, Novartis and **BYRON ARANA**, DNDi




Among the Neglected Tropical Diseases (NTDs), leishmaniasis – with all its clinical forms: visceral, cutaneous and muco-cutaneous – is a devastating condition for patients and adds a significant burden on the already stretched healthcare systems. The World Health Organization (WHO) road map for neglected tropical diseases 2021-2030, through its strategic framework for integrated control and management of skin-related neglected tropical diseases, has set specific targets and strategies for the control of cutaneous leishmaniasis (CL) worldwide. Besides improving detection, clinical diagnosis, reporting and treatment of detected cases of CL in endemic countries, one of the key actions identified is the development of novel oral or topical treatments to be used at primary health care level.

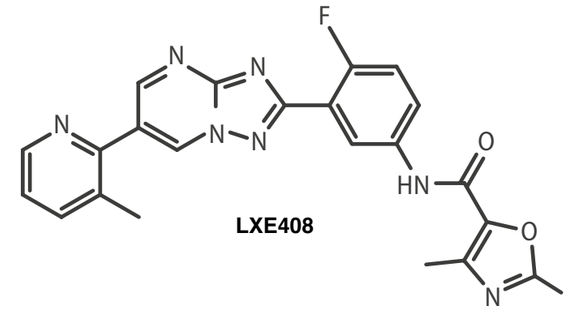
DNDi and its partners are collaborating to identify and advance the development of safe, effective, and affordable treatments for people with neglected diseases, including leishmaniasis and Chagas disease. LXE408 is a novel compound, discovered at Novartis with financial support from Wellcome (Khare et al. 2016). LXE408 is a first-in-class, selective kinetoplastid proteasome inhibitor, a potential drug candidate for the treatment of visceral and cutaneous leishmaniasis and also Chagas disease (Khare et al. 2016).

In preclinical *in vitro* models, LXE408 has shown to be highly selective in inhibiting the chymotrypsin-like activity of kinetoplastid 20S proteasome, and is potent against all *Leishmania* and *Trypanosoma cruzi* species tested. By inhibiting chymotrypsin-like activity, LXE408 interacts selectively with highly conserved subunits of kinetoplastid 20S proteasome (subunits b4 and b5) and does not inhibit human 20S proteasome. Hence LXE408 has the potential to be safe and effective across a large variety of *Leishmania* sp., including *L. donovani, L. infantum, L. major* and *L. braziliensis*. Further, LXE408 is effective in various *in vivo* models, including visceral leishmaniasis (VL) and old world cutaneous leishmaniasis: mouse model of VL (*L. donovani*), mouse lesion suppression model (*L. major*), mouse lesion cure model (*L. major*) (Nagle et al. 2020).

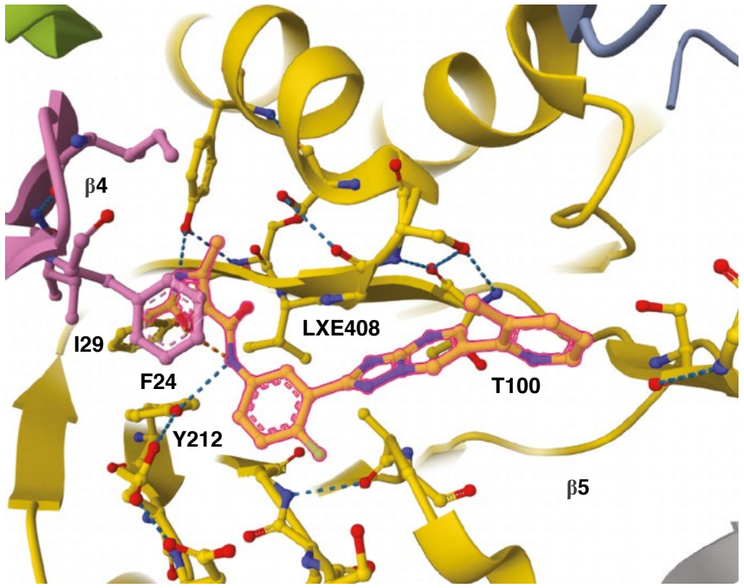
Safety evidence from a Phase 1 multiple ascending dose study in healthy volunteers supports the continuation of LXE408 to Phase 2 (Segal, 2022). LXE408 is currently being studied in Phase 2 clinical trials for visceral leishmaniasis (Kala-Azar) in India (clinical trials.gov NCT05593666) and Ethiopia (clinical trials.gov NCT05957978), with results expected in 2025. LXE408 has the potential to be a novel oral drug for the treatment of CL across all endemic areas and can add to the existing therapeutic arsenal against this debilitating disease. Building on the experience gained from the VL studies, DNDi and Novartis are collaborating to advance development of LXE408 for cutaneous leishmaniasis as well.

In the case of a complex disease such as CL, with highly heterogeneous clinical manifestations and large parasite diversity, the development of harmonized clinical trial methodologies is critical (Olliaro et al. 2018). The redeLEISH clinical research network is a valuable resource, with capacities for clinical evaluation across a range of sites. Leveraging the existing scientific expertise and research platforms, the successful development of a new promising asset will further benefit from an integrated collaborative approach of all stakeholders, with innovative partnerships between the public and private sectors. •

High-resolution cryo-EM structure of the *L. tarentolae* 20S proteasome in complex with LXE408



LXE408



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Segal F, *et al.* Oral presentation at WorldLeish7, Cartagena, Colombia, August 2, 2022

A Phase II, Multicentre, Randomized, Two-arm Blinded Study to Assess the Efficacy and Safety of Two LXE408 Regimens for Treatment of Patients With Primary Visceral Leishmaniasis. Available at: https://clinicaltrials.gov/study/NCT05593666 (accessed 29 Jan 2024).

LXE408 for Treatment of Visceral Leishmaniasis in Ethiopia, a Proof of Concept Study. Available at: https://clinicaltrials.gov/study/NCT05957978 (accessed 29 Jan 2024).

ATI-1801 FROM APPILI THERAPEUTICS: TOWARDS A SAFE AND EFFECTIVE TREATMENT FOR CUTANEOUS LEISHMANIASIS

DONALD CILLA, GARY NABORS, CARL GELHAUS, ARTHUR BARON and **JETSUDA AREEPHONG**, Appili Therapeutics Inc and **MENA ABDEL-NOUR**, Appili Therapeutics Inc and Bloom Burton & Co

Cutaneous leishmaniasis (CL) is the most common form of leishmaniasis, affecting hundreds of thousands of individuals globally. CL causes painful skin lesions that can persist for months to years and is associated with significant social stigma due to scarring and disfigurement that often occurs because of the disease. Unfortunately, current therapies for the treatment of CL, while moderately efficacious, are invasive, toxic, and may require hospitalization for administration. Furthermore, the mainstays of CL treatment are injectables, including amphotericin B; pentavalent antimonials, which are painful when administered; and oral miltefosine, which is expensive and therefore inaccessible to patients in the developing world that need them.

It is clear that safer and more easily administered treatments are needed for CL. A simple solution is a topical cream that could reduce systemic exposure to the drug while directly treating CL lesions. Topical creams have the dual benefit of limiting the potential for toxicity and being amenable to self-administration, as opposed to requiring daily injections administered by trained medical personnel.

Appili Therapeutics is working to bring one such solution to CL patients, a topical paromomycin cream (ATI-1801). Appili acquired ATI-1801 from the U.S. Army Medical Materiel Development Activity (USAMMDA) in 2019. At the time of acquisition, USAMMDA had completed a full clinical development program which included Phase 1 safety studies demonstrating tolerability, and Phase 2 efficacy studies in Colombia, France, and Tunisia, which showed superiority to placebo. Additional Phase 2 studies conducted in Peru and Panama demonstrated that ATI-1801 had comparable efficacy to a paromomycin-gentamicin combination cream. Phase 3 efficacy studies were conducted with 399 patients in Panama and 375 patients in Tunisia and demonstrated a clinical cure rate of 77.8% and 82% respectively. These studies suggested that ATI-1801 would be effective at treating both old-world CL and new-world CL.

Appili's goal is to facilitate broad access to ATI-1801 for CL patients worldwide. We are planning on working with World Health Organization (WHO) to include ATI-1801 for distribution to endemic countries, which have the greatest need to access the WHO's Prequalified Essential Medicines program. It is easier to navigate this process for products that have been approved by stringent regulatory authorities, such as the FDA.

Appili has identified a new manufacturer for ATI-1801 and is in the process of transferring the technology to them. Because there is a new manufacturer, Appili is working with the FDA on options to demonstrate the consistency of this product with the prior manufacturer. This may involve *in vitro* experiments and/or clinical studies to qualify the new manufacturer. In all scenarios, Appili is seeking partnerships and sources of funding to support advanced product development.

Once ATI-1801 is approved by the FDA, Appili will work with WHO to distribute it to CL-endemic areas in partnership with NGOs, local ministries of health and pharmaceutical companies, thus bringing a safe and effective treatment for CL to those who need it. •

LABORATORY GUIDELINES FOR DIAGNOSIS AND QUANTIFICATION OF THE PARASITE LOAD OF CUTANEOUS LEISHMANIASIS IN THE AMERICAS

OTACILIO C. MOREIRA, Molecular Analysis Platform, Virology and Molecular Parasitology Laboratory, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, **ANA NILCE SILVEIRA ELKHOURY**, Pan American Health Organization, Rio de Janeiro, Brazil and **ELISA CUPOLILLO**, Leishmaniasis Research Laboratory, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil

Cutaneous leishmaniasis (CL) is the most prevalent manifestation of leishmaniasis, with clinical polymorphism ranging from the presence of papules to multiple ulcers that affect both the skin and mucous membranes, resulting in permanent scars and serious disabilities. This disease, marked by its disfiguring and stigmatizing nature, often causes a psychosocial and economic impact on the communities that are affected.

In 2022, more than 35,000 cases of CL were reported in the Americas, with more than 60% of cases concentrated in Brazil, Colombia and Peru. Despite a downward trend in the number of cases over the last 20 years, in 2022 there was an increase in the incidence of the disease compared to the previous year (PAHO, 2023).

Diagnosis has emerged as one of the key challenges for effective control of the disease as a public health problem. The availability of diagnostic tools for CL becomes crucial to achieve the goals outlined in the WHO Roadmap for Neglected Tropical Diseases (NTDs) from 2021 to 2030, which sets out for the detection of 85% of all CL cases, followed by the treatment of 95% of reported cases (WHO, 2020).

In 2022, there was an improvement in the diagnosis of CL and mucosal leishmaniasis in the region of the Americas, with 86.2% of cases being diagnosed by laboratory methods, representing 7% more than the previous year (PAHO, 2023). In this context, the Pan American Health Organization (PAHO) is leading an initiative involving research centers from various CL endemic countries in the region. The aim is to develop a consensual, standardized and validated approach for the parasitological diagnosis of the disease using the qPCR technique, overcoming the low sensitivity of the available parasitological methods and helping the various countries of the Americas to improve their capacity and expand the laboratory diagnosis of the disease. The project involves the collaboration of seven groups from Argentina, Bolivia, Mexico, Panama, Peru and two from Brazil. »

Meeting with experts:

Defining qPCR as an approach to improve the diagnosis of cutaneous leishmaniasis in the Americas

Nov **2014**

Dez **2016**

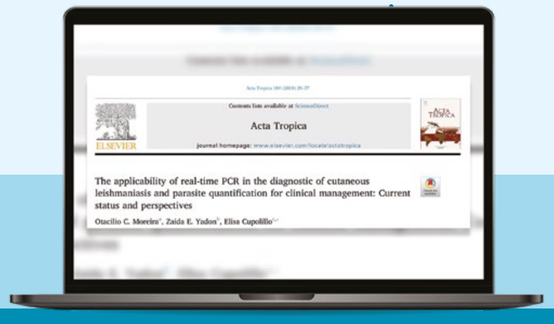
I International Workshop on the Standardization of a Real-Time PCR Assay for the Quantification of Parasite Load for the Management of Cutaneous Leishmaniasis in the Americas: University of Antioquia, Medellín - Colombia

2017

Planning activities and standardizing molecular assays

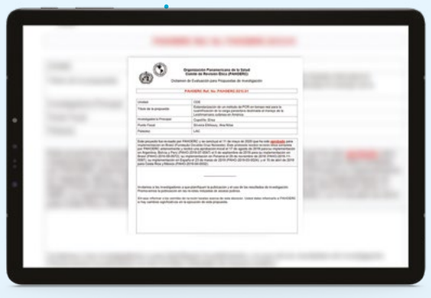
2018

Review of molecular methods used as a tool for the diagnosis of cutaneous leishmaniasis



2018 2020

Submission/Approval of the Project to the PAHOERC Ethics Committee and local committees



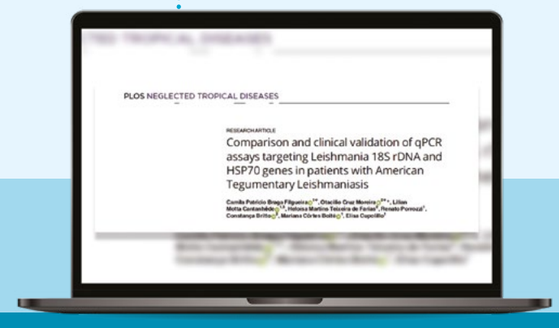
2020

Transportation of the samples to the Central Laboratory (Fiocruz-RJ) and start of the multicenter validation of the qPCR assays

Creation of sample panel, sending to all participating laboratories and return of results

2021 2022

Analytical validation and preliminary clinical validation of qPCR 18SrDNA and qPCR hsp70 for the diagnosis of cutaneous leishmaniasis



2023

Final analysis of the results and establishment of the consensus methodology for the molecular diagnosis of cutaneous leishmaniasis in the Americas

II International Workshop on the Standardization of a Real-Time PCR Assay for the Quantification of Parasite Load for the Management of Cutaneous Leishmaniasis in the Americas: Fiocruz RJ

Since the efficacy of PCR in the diagnosis of CL was demonstrated, various methodological approaches have been suggested and experimented with, including both conventional PCR (cPCR) and quantitative real-time PCR (qPCR). However, this is the first initiative to standardize and validate a specific methodology for diagnosing the disease, which allows for consensus building on protocols and molecular targets that can be used in an improved way to support clinical diagnosis.

The qPCR assays were standardized by Fiocruz, Rio de Janeiro (Central Laboratory), using DNA extracted from promastigotes of various *Leishmania* species, followed by preliminary clinical validation. This validation was carried out on samples collected from patients with clinical suspicion of CL, with or without confirmation of the parasitological diagnosis. Multiplex qPCR assays were carried out targeting human RNAse P (endogenous internal control), 18S rDNA and hsp70 in the parasite. A significant correlation in parasite quantification was observed between the two targets used. When considering microscopic examination as the standard reference for parasitological diagnosis, the results of the two simultaneous qPCR assays showed a sensitivity of 98.5%. Furthermore, by sequentially analyzing the results of the qPCR for 18S rDNA and, subsequently, the qPCR for hsp70, maximum specificity (100%) was obtained (Filgueira et al., 2020).

The following stage was multicentre clinical validation using the standardized methodology for three *Leishmania* spp targets: hsp70, 18SrDNA and kDNA. At least fifteen samples of skin lesions from patients with CL were collected by each of the participating laboratories, for a total of 128 samples. DNA from each sample was extracted under standardized conditions, using an exogenous internal control, and sent blindly to the Central Laboratory, where it was subjected to qPCR assays. The results showed positivity ranging from 67-100% and 80-100% for the hsp70 and 18SrDNA targets, respectively. The kDNA assays showed approximately 30% positivity for the samples from Mexico, which raises some possibilities that indicate limitations to using this target for the entire region due to the heterogeneity of this target between species of the *Leishmania* and *Viannia* subgenera. The best sensitivity was obtained for the 18SrDNA target, which was considered almost perfect. A specificity of 100% was obtained for this target and also for hsp70. There was a drop in qPCR positivity for samples with Ct for RNAse P of 28 and higher, indicating that as well as acting as an endogenous control for the reaction, the result for RNAse P allows for a better interpretation of the results obtained for the *Leishmania* spp targets.

A panel of 68 samples was then built from the samples sent in by the various participating groups and reference samples, which were sent blindly to each group to carry out the tests. The results showed good reproducibility compared to the results obtained by the Central Laboratory. In general, there was a good correlation and agreement between the parasite load determined by the Central Laboratory and the other participating laboratories.

After a 9-year effort, the results were presented and discussed during the II International Workshop on the Standardization of a Real-Time PCR Assay for the Quantification of Parasite Load for the Management of Cutaneous Leishmaniasis in the Americas, attended by specialists from the six countries participating in the initiative. Everyone had the opportunity to perform the protocols during the workshop so that any doubts could be clarified. In the end, considering all the results obtained, the group unanimously concluded that the qPCR protocol targeting the 18SrDNA region of *Leishmania* spp should be used in the molecular diagnosis of CL. Although it was not the focus of this initiative, other studies indicate that this protocol can be applied in the diagnosis of the various clinical manifestations of CL and could be an important tool for the diagnosis of ML.

To conclude the study, samples of skin lesions from other etiologies are being analyzed to improve the evaluation of the specificity of the tests. The results are being organized for scientific publication. As this is the only validated methodology for the molecular diagnosis of CL in the Americas, a technical report with the detailed methodology has been drawn up to be shared with national laboratories, so that the protocol can be incorporated to the flow of laboratory diagnosis of CL in the different countries of the region.

All the scientific and technical activities are being coordinated by the Leishmaniasis Research Laboratory and the Molecular Analysis Platform – Virology and Molecular Parasitology Laboratory, Oswaldo Cruz Foundation, Brazil, with the support of PAHO and DNDi. •

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ADVANCING POINT-OF-CARE MOLECULAR DIAGNOSTICS FOR SKIN-RELATED NEGLECTED TROPICAL DISEASE

DR SUNDEEP CHAITANYA VEDITHI,
American Leprosy Missions & University of Cambridge

American Leprosy Missions (ALM) is an international organization committed to alleviating the impact of Neglected Tropical Diseases (NTDs), known for causing disabilities and chronic complications. ALM's mission encompasses not only physical healing but also the restoration of dignity and hope in affected individuals. The organization leads in the development and investment in innovative projects, systems, technologies, and tools aimed at fostering sustainable, positive change and expediting the discovery and application of groundbreaking solutions for the treatment, care, and eventual eradication of high-morbidity NTDs.

In 2019, a pivotal partnership was established between ALM and Biomeme Inc. This collaboration originated when the Director of Research and Innovation at ALM attended the Mycobacteriology Conference at Colorado State University, where the potential of Biomeme's technology for detecting Mycobacterial infections was explored. Consequently, ALM and Biomeme Inc. entered into a bio-development and revenue-sharing agreement to research and develop cost-effective, field-friendly, point-of-need qPCR-based molecular diagnostics for NTDs, suitable for use in hospitals and diagnostic centers in NTD-prevalent tropical countries. By 2020, ALM, in collaboration with Kumasi Centre for Collaborative Research in Ghana, the Schieffelin Institute for Leprosy Research in India and the Colorado State University in the USA, had developed independent molecular assays capable of detecting Buruli ulcer (BU) or leprosy in clinical isolates. »

The Biomeme Franklin™ qPCR system represents a significant advancement in molecular diagnostics. This time and cost-effective, field-compatible system operates at ambient temperature and is ideally suited for real-time qPCR-based detection of pathogen nucleic acids at the point-of-care settings. The battery-operated system features DNA extraction reagents in a closed cartridge, enabling rapid nucleic acid extraction from skin samples without risk of cross-contamination. The pre-mixed qPCR reagents are lyophilized, ensuring stable, extended shelf life at ambient temperatures (15-45° C). The process from sample to result takes approximately one hour and requires minimal training, making diagnosis simple, accurate, and feasible in remote health facilities. The implementation of these qPCR systems in peripheral health settings in leprosy-endemic countries is crucial for confirmatory diagnosis, given the declining clinical expertise in leprosy. Preliminary proof-of-concept studies for the Biomeme Leprosy Assay, conducted with partners in India, Tanzania and the USA, demonstrated a sensitivity greater than 95% for Paucibacillary and Multibacillary leprosy, and a specificity of >99%. We currently aim to conduct a multicentric evaluation of the performance of these Biomeme Leprosy qPCR assays to confirm their efficacy and test the feasibility of implementation at the periphery of the health system. The Biomeme Buruli Ulcer assays have outperformed standard qPCR systems in reference diagnostic labs, with ongoing evaluations for the leprosy assays.

Initial findings reveal a 98% sensitivity in detecting standard bacterial DNA from as low as three bacterial cells in skin scraping tissue samples for leprosy, and 95% sensitivity for BU, maintaining a specificity of 100% for both assays. This performance is attributed to the selection of pathogen-specific genes, as detailed in published literature. A similar approach was successfully implemented in Ghana for Covid-19 diagnosis during 2020 and 2021, using Biomeme qPCR assays in mobile diagnostic facilities, proving effective in expanding coverage for detection and referrals. ALM facilitated the supply of qPCR machines and reagents for this initiative.

Skin NTDs rank as the third most prevalent cause of illness worldwide and are among the top ten causes of disability according to the World Health Organization (WHO). They require similar detection and management approaches, with molecular diagnostics being crucial for identifying five diseases: leprosy, Buruli ulcer, yaws, dermal leishmaniasis, and mycetoma. With funding from the Anesvad Foundation and the Raoul Follereau Foundation, ALM will now utilize its expertise in diagnostic assay development to create a multiplex assay for simultaneous detection of these five skin NTDs using the Biomeme system.

Selected research laboratories and reference diagnostic centers in Benin, Cameroon, Côte d'Ivoire, Ghana, Sudan, Spain and the Netherlands will develop and evaluate Biomeme Multiplex skin NTD assays. Many of these countries are endemic for the targeted skin NTDs, with several of the laboratories being part of the Skin NTD Laboratory Network (Skin NTD LAB-NET), a consortium of laboratories mainly from West African countries, founded by WHO and funded by ALM, Anesvad and the Raoul Follereau Foundation. Coordinated by Centre Pasteur Cameroon as a partner lab, this network oversees the External Quality Assessment (EQA) program for diagnosing BU and yaws within member labs. It ensures timely collection, collation, and dissemination of results to hospitals, national programs, ministries of health, and patients, exemplifying ALM's commitment to enhancing diagnostic capabilities and improving health outcomes in regions burdened by NTDs.

For the development and evaluation of Biomeme *Leishmania* assays using genus-specific primers and probes, we collaborated with the Leishmaniasis and Chagas Disease Unit of the National Center for Microbiology – Instituto de Salud Carlos III in Spain (ISC-III). As the National Reference Laboratory for *Leishmania* infections, ISC-III bears the responsibility of supporting the Spanish National Health System in controlling *Leishmania* and *Trypanosoma cruzi* infections. This center specializes in the diagnosis and analysis of the immune response to both natural and experimental canine leishmaniasis. They have dedicated efforts to developing an experimental infection model in dogs, successfully used in evaluating *Leishmania* antigens and in laboratory trials of vaccines against canine leishmaniasis. ISC-III will develop Biomeme genus-specific *Leishmania* assays and subsequently enable the Noguchi Memorial Institute for Medical Research in Accra, Ghana, to conduct analytical and clinical validation. Furthermore, ISC-III will potentially contribute to the development of species-specific Biomeme multiplex qPCR assays for leishmaniasis, suited to different geographies. This task will be undertaken in addition to the earlier stated multiplex skin NTD assays. •

FORMALIZING THE BRAZILIAN ASSOCIATION OF PEOPLE WITH LEISHMANIASIS - ABRAPLEISH

ALEX LAGO, **TANIELE CUSTÓDIO**, **ELY AMORA**, and **ANA PAULA FERREIRA**, ABRAPLEISH

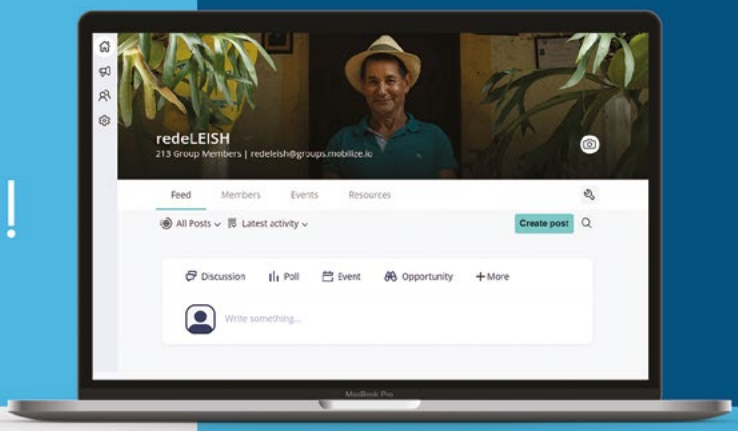
Leishmaniasis is caused by protozoa of the genus *Leishmania* and transmitted by phlebotomine sandflies, which include more than 20 species. It is an infectious, non-contagious disease that affects thousands of people in various regions of the world. The disease is divided into two clinical forms: cutaneous leishmaniasis and visceral leishmaniasis, which mainly affect people who are more socioeconomically vulnerable.

The Brazilian Association of People with Leishmaniasis (ABRAPLEISH) was created with the aim of advocating for the necessary conditions for people living with leishmaniasis, who often live in neglected communities and whose access to health care is precarious and limited by public authorities. The association aims to mobilize organizations so they can better support people affected by the disease. In addition, most health units do not offer excellent treatment, diagnosis or social assistance. In view of all this negligence on the part of the public authorities in caring for people affected by leishmaniasis, the ECLIPSE research group from Universidade Federal da Bahia (UFBA)'s Institute of Collective Health (ICS) has been working in the leishmaniasis-endemic area of Bahia. This is an important endemic area, where more than 2,000 cases of tegumentary leishmaniasis are recorded every year. Thus, in partnership with DNDi, the idea arose of inviting people from the community to set up an association.

Diogo Galvão, regional access consultant from DNDi, was present in the leishmaniasis-endemic area of Bahia and spoke to several people in the community who were directly or indirectly affected by the disease. He explained the importance of revitalizing this association and its impact on the public sphere, and some people in the community agreed. During a few months, online meetings were held to better detail the refoundation process of the association, which had been created in 2018 by Moacir Antonio Zini, who left us in 2020. Some people were invited to take part in events, such as the Neglected Diseases Forum held in Salvador, Bahia in 2023. During these events, we realized the importance and magnitude of an association in social movements. Since then, we have been gradually developing our strength in order to fight for and achieve improvements for people who are still neglected by public authorities.

So we were able to start the formalization process of the association with the support of DNDi, ECLIPSE and groups from other neglected disease associations in Brazil. On November 14th 2023, association members Alex Lago, Taniele Custódio, Ana Paula Ferreira, Ely Amora and other people from the community signed the minutes of the ABRAPLEISH association, held in the city of Presidente Tancredo Neves/ Corte de Pedra, Bahia. •

Join the redLEISH webforum!



Access the online platform together with **experts from all over Latin America** to receive and share **information on leishmaniasis research**

The forum works as a social network in which you can also receive updates by email. Members can interact and post news, facilitating communication among collaborators. We encourage the sharing of documents and scientific papers, the promotion of events and debate, and the possibility of asking questions and making new contacts.

Register using the **QR code**

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DNDi Latin America
Rua São José, 70, sala 601- CEP 20010-020 - Rio de Janeiro, RJ - Brazil
Tel: +55 21 2529-0400 | www.dndial.org

DNDi Global Headquarters
15 Chemin Camille-Vidart 1202 - Geneva - Switzerland
Tel: +41 22 906 9230 | Fax: +41 22 906 9231 | www.dndi.org