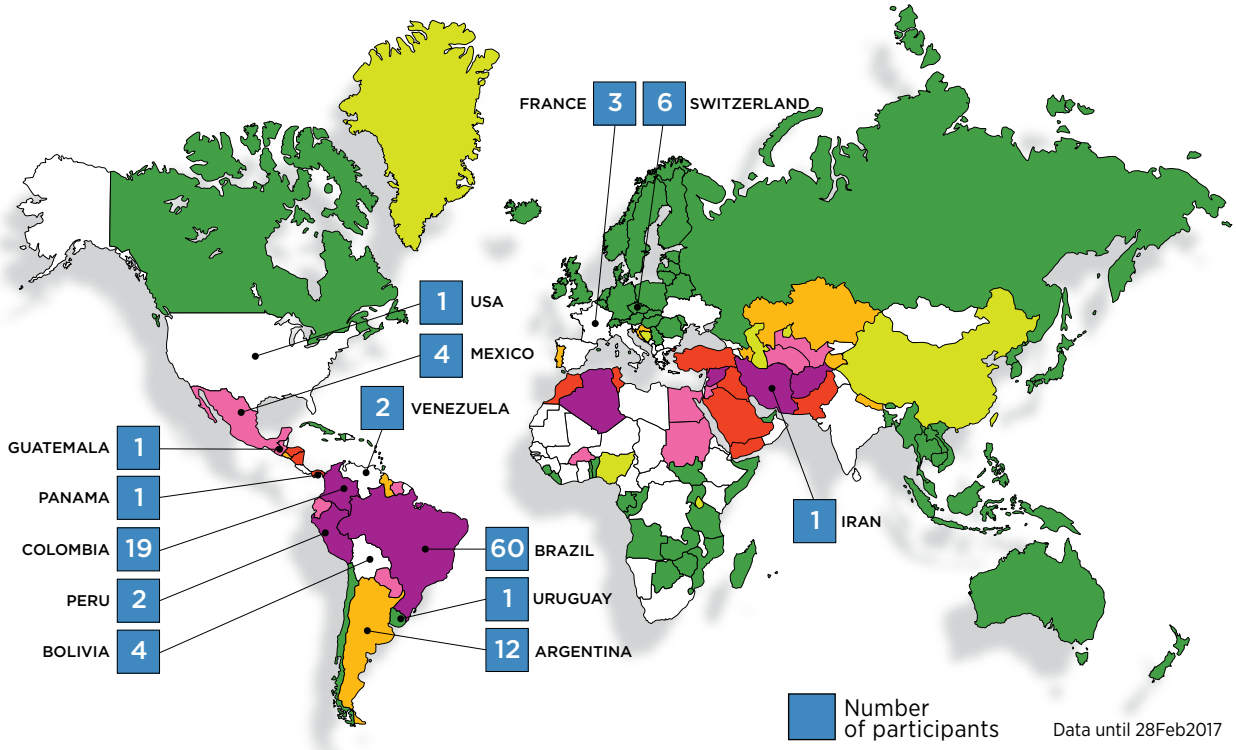
InfoLEISH

redeLEISH newsletter - 2nd edition

May 2017

GEOGRAPHICAL DISTRIBUTION OF REDELEISH PARTICIPANTS & STATUS OF ENDEMICITY OF CUTANEOUS LEISHMANIASIS[1]



[1] Based on WHO epidemiological situation, 2013. Available in: http://www.who.int/leishmaniasis/burden/Leishmaniasis_Burden_distribution_VL_CL_2013.pdf?ua=1

NUMBER OF NEW CL CASES REPORTED, 2013:

0 (zero) | < 100 | 100 - 999 | 1000 - 4999 | ≥ 5000 | No autochthonous cases reported | No data

editorial

THE IMPORTANCE OF WORLDLEISH 6

The WHO Collaborating Centre for Leishmaniasis, Instituto de Salud Carlos III, Madrid, and the Drugs for Neglected Diseases *initiative*, Geneva are co-organizing the WorldLeish 6 Congress to be held in Toledo, Spain, from 16 to 20 May 2017.

WorldLeish represents a unique opportunity, which comes every four years, and brings together experts, professionals, researchers of different fields and health authorities to discuss a variety of topics related to leishmaniasis, ranging from basic research to access to drugs and operational activities for the control of the disease. The richness and uniqueness of WorldLeish also comes with the diversity of institutions represented, including individuals from private and public academic institutions, charitable organizations, advocacy groups, government officials, etc. Last but not least, WorldLeish 6 is an exciting opportunity to network, share knowledge and, why not, doing some tourism in the host city of Toledo.

In the context of WorldLeish 6, it is our pleasure to invite you to attend the 4th redeLEISH meeting for researchers and collaborators on cutaneous leishmaniasis.

As in previous redeLEISH meetings, researchers will have the opportunity to exchange information on the different projects and initiatives that redeLEISH is closely following up and hopefully provide an opportunity to launch new research ideas. The meeting will also offer a platform to reinforce and further promote partnerships to address forgotten issues such as mucosal or mucocutaneous leishmaniasis, which affect thousands of people in the New World and is a rising problem in the Old World as well. RedeLEISH aims to continue expanding and hopefully include more and more researchers from other countries beyond Latin America, so we invite everyone interested in cutaneous leishmaniasis to attend the 4th meeting.

We welcome you to Toledo, and will make sure that you have a memorable WL6 and 4th redeLEISH meeting.

BYRON ARANA
Head of Cutaneous Leishmaniasis
DNDi – Geneva, Switzerland

summary

CLINICAL AND THERAPEUTIC UPDATE ON MUCOSAL LEISHMANIASIS

DR. ALEJANDRO LLANOS-CUENTAS
Institute of Tropical Medicine
Alexander von Humboldt,
Peruvian University Cayetano Heredia

Leishmaniasis remains a serious public health problem despite all the technological advances in recent decades. The number of cases has not changed significantly in the last 50 years. The increase or decrease in number of cases basically depends on factors which are external to health programs.

There are three clinical forms of leishmaniasis described: cutaneous, mucocutaneous (or mucosal), and visceral. Tegumentary leishmaniasis is a spectral disease, where mucosal leishmaniasis (ML) is its extreme form. Mucosal involvement is usually due to a metastatic mechanism (<2% by contiguity) and in most cases the disease starts in the nasal septum and turbinates. Subsequently, it extends to the rhinopharynx, pharynx, uvula, soft palate and in severe instances moves to the epiglottis, vocal cords, subglottic region, trachea and even to bronchi. The most frequently involved species is *L. (V.) braziliensis* and to a lesser extent *L. (V.) guyanensis*; however, although rarely observed, any *Leishmania* species may cause mucosal involvement.

The treatment of choice for ML remains pentavalent antimonials (Sb^{5+}) at the dose of 20 mg Sb^{5+}/kg/day for 30 days (as recommended by WHO); however, the therapeutic response varies depending on the severity of the disease. In a series of 81 patients presenting with ML, with manifestations in the nasal and oral cavity (mild group), treated with Sb^{5+} (as recommended by WHO), the efficacy rate was 84.5%. When the disease manifested in the epiglottis (moderate group), the cure rate decreased to 40.9% and when it involved the vocal cords (severe group), the cure rate was only 7.1%. In this severe group, the disease also manifested in the subglottic region (70%), trachea (30%) and bronchi (14%). Evidently, patients with moderate and severe forms of ML should not be considered for the treatment with Sb^{5+}. Further studies have confirmed that severe forms of ML show poor therapeutic response to Sb^{5+}.

An alternative drug is amphotericin B deoxycholate (AMPB, dose 0.7mg/kg/day), however the total dose to be used has not been clearly established. In our experience most patients with ML achieve cure with a cumulative dose of 25mg (approximately 42 doses); however, patients with the disease manifesting in the trachea and bronchi require higher doses, which is determined by a bronchoscopic evaluation (patients must be treated until they reach the criteria of cure).

The limitation in the use of AMPB is the systemic toxicity, especially renal, plus the fact that clinicians have poor experience in the management of this drug. Preventative alternatives have been developed to reduce glomerular (use of saline pre-infusion) and tubular damage (early replacement of Mg and K), but there are no detailed guidelines for physicians to use this medication in a better way.

In some countries, the use of liposomal amphotericin B has become more common (AMPBL); however, with inadequate doses and treatment periods. In a study carried out by Llanos *et al*. (not published) comparing AMPBL (3mg/kg/daily for 21 days) *versus* AMPB (cumulative total dose of 25 mg/kg), the cure rate in severe cases of ML was 90% (9/10) in patients treated with AMPBL against 82.3% (14/17) in the group treated with AMPB (p>0.52). In the moderate ML group, the cure rate was similar: 93.3% for AMPBL (14/15) and 93.3% for AMPB (28/30). The difference observed in this study was in the occurrence of adverse events, only one patient from the AMPBL group had the treatment temporarily suspended (2%, 1/50) compared to 23% (6/26) of patients from the AMPB group.

Another therapeutic alternative is the combination of Sb^{5+} with pentoxifylline (400 mg tid) which shows an increment in the cure rate. A recent evaluation of the efficacy of this combination in 205 patients with ML (carried out in Lima, Peru) has shown an increase in the cure rate of 61% in the group treated with Sb^{5+} monotherapy versus 79% (p=0.011) in the group treated with Sb^{5+} plus pentoxifylline.

Miltefosine is an oral drug (2.5mg/kg/day for 28 days) that has shown to be effective in patients with ML in Bolivia; however, the Peruvian experience was not very positive with this medication, as only 1 patient was cured out of 10 treated.

In summary, therapeutic regimens for ML remain lengthy and with frequent adverse events, which require specialized management and high costs. The use of AmBisome® as an alternative must be evaluated in a cost-benefit basis in two contexts: in endemic developing countries *versus* developed countries.

THE IMPORTANCE OF SEEKING THERAPEUTIC SOLUTIONS FOR MUCOSAL LEISHMANIASIS IN LATIN AMERICA

EDGAR CARVALHO[1, 2] and PAULO MACHADO[1]
[1]Department of Immunology, University Hospital Professor Edgar Santos - Federal University of Bahia
[2]Gonçalo Moniz Research Institute - Oswaldo Cruz Foundation

Although more commonly known as mucocutaneous leishmaniasis (MCL), the term mucosal leishmaniasis (ML) is more accurate to define the disease, because cutaneous and mucosal lesions rarely occur concomitantly and more than 10% of people with ML show no previous history of cutaneous leishmaniasis (CL). ML is caused predominantly by *Leishmania Viannia braziliensis*, but may also be caused by other species, such as *L. amazonensis* and *L. guyanensis*. The disease mainly affects the nasal mucosa, but the palate, pharynx, and larynx can also be affected. In some cases, mucosal involvement may lead to destruction of the face structure, causing respiratory failure associated with death, and to greater difficulty to respond to treatment, that results in increased morbidity and mortality, hence the importance of ML.

The pathogenesis of ML and the treatment failure observed in some cases are related to parasite and host factors. Isolates of *L. brasiliensis* associated with mucosal disease differ genetically from CL isolates, and antigens from ML isolates induce greater inflammatory response than those prepared with CL isolates. The excessive inflammatory response is characterized by an exacerbated production of pro-inflammatory cytokines such as CXCL-9, CXCL-10, TNF and IFN-Y. Because this response is not properly modulated by regulatory cytokines, the inflammatory process persists and leads to tissue damage. It is also known that the presence of a RNA virus (LRV1) in *leishmania* isolates contributes to the severity of tegumentary leishmaniasis and interferes in the therapeutic response.

Because ML occurs predominantly in Latin America, pentavalent antimonial is the drug most commonly used for its treatment. Nevertheless, therapeutic failure ranges from 40% to 50% at the dose of 20mg/kg/body weight for 30 days. Based on evidences that the inflammatory response is involved in the pathogenesis of tegumentary leishmaniasis, the association of antileishmanial drugs with immunomodulatory agents has been used in the treatment of this disease. In case of ML, the association of antimonial with pentoxifylline, a drug able to decrease TNF production, was more effective than the antimonial alone, significantly reducing time to cure, and healing patients who were refractory to antimonial treatment. Besides, the high rate of therapeutic failure, the need for parenteral administration of the drug and the adverse reactions to antimonials are limiting factors for its use. In addition, although the treatment period is of 30 days, the definition of cure or therapeutic failure is only confirmed 60 days after the end of treatment.

Miltefosine is the only oral drug proven to be efficacious against ML and it has been successfully used in the treatment of this disease, although, therapeutic failure has also been reported. In a clinical trial, therapeutic failure was observed in 17% of subjects with mild manifestation of the disease, characterized only by the involvement of the nasal mucosa, and 42% in those patients with severe condition, characterized by the involvement of pharynx, and larynx. Amphotericin B with total dose ranging from 1.5 to 2.5 g is efficacious in more than 90% of patients and it also cures refractory cases to antimonial. Nevertheless, its toxicity, mainly related to kidney failure, is a limiting factor for its use. Additionally, its administration proves to be difficult in rural areas, and, in Bolivia, therapeutic failure was observed in about 50% of patients, even when using an average dosage of 45 mg/kg of body weight.

Many hurdles must be overcome in order to identify a drug that shows high efficacy and low toxicity for the treatment of ML. Besides the low priority to invest in robust clinical trials, many other factors may influence the outcomes for leishmaniasis as a whole, and particularly for ML. Most of the published studies are not randomized or controlled, it is difficult to establish the duration of the mucosal disease, and previous history of CL treatment may also influence the therapeutic response as well as the severity of the mucosal disease. Furthermore, ML presents a wide and heterogeneous spectrum of clinical manifestations, from small and thin granulations in the nasal septum to the involvement of the functions of pharynx and larynx, going through superficial to deep ulcers and even perforation of the nasal septum. The variability of this disease needs to be considered when determining the right choice of treatment.

It is known that, amongst those drugs commonly available for the treatment of leishmaniasis, the best therapeutic option is liposomal amphotericin B. There are several evidences of success when using this drug for the treatment of ML. However, because these studies are not controlled and include a small number of patients, there is no definition until now about the total dose that should be used. Therefore, more incentive and funding are needed to perform well-structured clinical trials with liposomal amphotericin B and other drugs, in order to develop more effective and safer treatments for ML.

Jorge Ferreira was patient of mucosal leishmaniasis in Dr. Jackson Lopes da Costa Center in Bahia, Brazil

Thermotherapy machine

USE OF COMBINED THERAPIES FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS

BYRON ARANA
Head of Cutaneous Leishmaniasis
- DND*i* Switzerland

Given the clinical spectrum of cutaneous leishmaniasis (CL) it is generally accepted that its treatment should be based on the clinical presentation: local therapies for patients with small and few lesions; systemic treatments, preferable oral drugs, for subjects suffering from numerous or large lesions, or subjects with lesions which are potentially disfiguring, disabling or located in areas which make local therapy impossible (face, joints, toes, fingers, eyelids, lips, ears); whilst subjects with leishmaniasis recidivans, diffuse CL, or Post-kala-azar dermal leishmaniasis (PKDL) should benefit from treatment with antileishmanial drugs in combination with an immune response modifier (IRM) to accelerate and enhance a Th-1 type immune response.

WHO recommendations are based on the causative species, geographical area and the clinical features of the disease and its recommendations varies from no treatment to topical or systemic approaches.

Even though progress has been made in the identification and preclinical development of compounds against *Leishmania* species causing cutaneous disease, in the short period of time (5-7 years) what is currently available will probably represent almost the entire therapeutic arsenal for the coming years, hence the need to explore ways to optimize the use of existing tools.

Drug combinations are commonly used for the treatment of different infectious diseases, including malaria, tuberculosis, leprosy, and visceral leishmaniasis. Several studies have also been conducted on CL testing different treatment approach combinations. A common problem in many of these trials however, is that the combinations included compounds or approaches for which there was no strong evidence of its effectiveness when administered alone.

Both miltefosine at doses of 2 mg/kg per day for 28 days and Thermotherapy single application at 50°C for 30 seconds, have shown to be effective for the treatment of CL (cure rates ~70% in both Old and New World) when used alone. In an effort to improve the efficacy of these two approaches when used in combination, DND*i* is conducting a phase II study aiming to determine the efficacy and safety of a combined therapy using thermotherapy (TT) (one application, 50°C for 30 seconds) + miltefosine, 2.5 mg/kg/day for 21 days for the treatment of uncomplicated CL in Peru and Colombia.

The advantages offered by testing the combination of thermotherapy plus miltefosine are the following: a) we are using two approaches that are currently recommended for use individually and for which there is good information regarding their efficacy and safety when used alone; b) the use of a topical plus a systemic treatment would hypothetically have an additive effect, since systemic treatment would eliminate those circulating or remaining parasites located in the periphery of the lesion that topical treatment fails to remove and which might be the cause of relapses; c) it offers the opportunity to increase the current cure rate reported with any other treatment approach available when used alone; d) it will reduce the length of treatment with miltefosine and hopefully the cost and rates of adverse events associated with 28 days of treatment with miltefosine alone. The selection of thermotherapy was due to its advantages: 1) high security profile; 2) requires only one application which ensures compliance with treatment; 3) easy to use in the field since the machine operates with batteries; 4) its effectiveness does not depend on the species of *Leishmania* causing the lesion as it is a physical measure.

The use of this combination could bring in a short period of time a better treatment option for a large proportion of individuals suffering from CL with hopefully a much better efficacy and safety profile than with the use of antimonials.

REGULATORY ASPECTS OF CONDUCTING THE COMBINATION THERAPY TRIAL FOR UNCOMPLICATED CUTANEOUS LEISHMANIASIS IN PERU AND COLOMBIA

MARINA BONI
Clinical Trial Manager
- leishmaniasis
DND*i* Latin America

WHAT IS THE BACKGROUND?

Similar to many countries in the world, both Peru and Colombia have two instances for approval in a clinical research setting: the Ethics Committees (EC) and the Regulatory Authorities (RA).

The RA in Peru is divided into two, the ethical and scientific approval of study (INS – Instituto Nacional de Salud) and the release of an import license, which authorizes the study medication to enter the country (DIGEMID – Dirección General de Medicamentos, Insumos y Drogas); whereas in Colombia, INVIMA (Instituto Nacional de Vigilancia de Medicamentos y Alimentos) provides not only the ethical and scientific approval for the conduct of the study but also the authorization to import the study medication.

WHAT IS THE PROBLEM?

Latin American countries are well known for their lengthy bureaucratic process of document review and approval in clinical research. The CL Combination study stumbled in two main obstacles prior to first patient being enrolled.

Study started off with a prospect of having the first patient enrolled by May 2016, but as a result of 1) the inability of finding appropriate resources for manufacturing a placebo IMP, and 2) regulatory delays, hence first patient enrolled into the study was in fact in December 2016 (in Peru).

WHY THE DELAYS?

The initial plan was to have a placebo controlled study, meaning that the two arms of the study would have been: thermotherapy (TT)+ miltefosine, compared to TT + matching placebo. After several attempts to find an appropriate clinical supplies' organization/ pharmaceutical partner in Latin America (we have looked in Brazil, Colombia and Peru) who could assist us in developing the matching placebo, we have realized that these countries do not possess any partners with enough expertise and/ or interest to perform such tasks.

Apart from manufacturing the matching placebo itself, several tests would have had to be run with the miltefosine to validate an adequate methodology in order to ensure the matching placebo did not contain any traces of miltefosine. In addition, the miltefosine itself would have had to be unpacked from its original blisters, the watermark of each capsule erased and all capsules completely re-blistered (so same blisters would have been used for matching placebo and miltefosine). That did not happen, and if it did, it would've caused the study further and further delays.

In this case, it would have been extremely helpful to have embarked on a pre-assessment in each country, at the time of the design of the protocol, in order to check whether there was any local expertise that could have assisted us to manufacture the placebo, re-blister the miltefosine and run all required quality control tests as per local and international regulations.

The other option would have been to use a clinical supplies organization outside of Latin America, however, from previous experience with other studies conducted at DND*i* this has proven to be a very costly and lengthy exercise.

Most of us agree that having a randomized placebo controlled trial is a very robust and statistically solid endeavor to be undertaken. However, in some cases, including this study, such design would have meant increased overall costs and lengthy timelines for start-up. Therefore, in order to keep the study moving along, it was decided to exclude the matching placebo from the design, considering that the adverse events of the miltefosine itself are so evident and widely known that investigators would have been able to identify patients who were in the miltefosine arm.

Considering we had to revise the entire protocol and informed consent forms, EC and RA approvals had to be obtained again. That itself took a couple of months. In general, the experience with the ECs both in Peru and Colombia were good, as they did keep with their scheduled meetings (although in Peru we had a few difficulties in obtaining approval letters with the adequate version dates for the documents approved). In Colombia though, we had a few 'hiccups' as new EC members were effective for the first round of protocol review, and misunderstandings resulted in another month's delay.

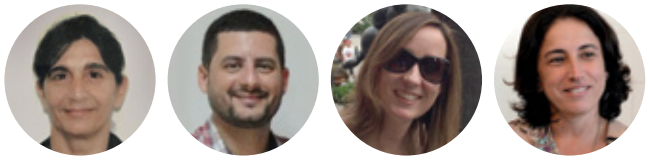
Over all, the RAs in both countries asked relevant questions, completely different from each other. In summary:

The Peruvian regulatory approval from INS came relatively soon after the questions were answered, however when it came to the release of the import license, that took much longer than expected. One issue was that the timelines for receiving an answer from DIGEMID were way outside of timelines provided. And, the other issue was that DIGEMID insisted on getting specific quality control (QC) documents that not even Knight Therapeutics Inc. had (pharma who donated the miltefosine to the study). After a bit of digging, we were able to locate the necessary QC documents and approval was obtained to bring miltefosine into Peru. First patient was enrolled on 10 December 2016.

In Colombia, it usually takes 2-3 months for the RA to approve a study and 5 more weeks for the answers we have provided to be reviewed. In our case, after all questions were answered, there was a problem with INVIMA's Information Technology system, which prevented their staff from receiving/ entering our answers into their system. So, to our frustration, the 5-week period would only start counting when all answers were in the system. And another month went by. INVIMA's approval is yet to be received as of mid-February 2017.

If we compare the start-up processes of a trial in developed countries against developing countries, there seems to be a big gap in terms of efficiency. Latin America is still a long way from having efficient processes which we can rely upon to obtain clinical research approvals in the expected times. There are a lot of people involved in the processes for the work to be done, and if one is unwilling or if a system does not work, that is where the bottleneck may be found. Problem solving skills and generating/ evaluating innovative and alternative solutions for the problems we encounter may be a place to start!

ZAIDA E. YADON[1], OTACILIO C. MOREIRA[2], LUIZA DE O. R. PEREIRA[3] and ELISA CUPOLILLO[3]
[1] Communicable Disease Research. Communicable Diseases and Health Analysis Department. Pan American Health Organization
[2] Laboratório de Biologia Molecular e Doenças Endêmicas, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil
[3] Laboratório de Pesquisas em Leishmanioses, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil



INTERNATIONAL WORKSHOP ON THE STANDARDIZATION OF A REAL TIME PCR ASSAY FOR THE QUANTIFICATION OF PARASITE LOAD FOR CUTANEOUS LEISHMANIASIS MANAGEMENT IN THE AMERICAS

Leishmaniasis is considered the most neglected tropical disease, according to the disability-adjusted life years (DALYs). Globally, around 12 million people are infected, and 350 million live in risk areas. The disease presents different clinical manifestations, as asymptomatic infections or the two most common forms: visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL).[1] The clinical manifestation of CL ranges from small-localized lesions to disseminated large ulcers all over the body. This clinical manifestation is associated with several *Leishmania* species in the New World (the Western Hemisphere), mainly *L. mexicana, L. amazonensis, L. braziliensis, L. guyanensis, L. panamensis and L. peruviana*, depending on the geographic region.

The diagnosis of the disease is performed by the combination of clinical, epidemiological, and parasitological tests. Parasitological diagnosis remains the gold standard and includes microscopic examination of smears or aspirates, histopathological examination of lesion biopsies, or culture of biopsy triturates or aspirates.[2]

Molecular parasitological approach for the diagnosis of cutaneous leishmaniasis and identification of the parasite has been in place since decades and has great potential to be applied directly in the clinical sample, avoiding the time-consuming isolation and cultivation of the parasite.

Since the beginning of the application of PCR to diagnose leishmaniasis, several methodologies were tested without consensus regarding protocols and molecular targets. It prevents data comparison, since each research group uses his own in-house protocol, even for sample preparation. Thus, the lack of standardization and validation of a consensus protocol for molecular diagnosis and parasite load estimation represents a need to conduct studies which look at the development of new drugs, epidemiological surveillance and routine clinical diagnosis.

In this context, the Communicable Disease Research Programme of the Pan American Health Organization promoted the standardization and validation of PCR for CL diagnosis and disease management across laboratories and countries. Accordingly, a project proposal was developed to validate and harmonize PCR methods during a workshop with the participation of experts from molecular biology laboratories of endemic areas working in PCR for CL. This workshop was financed by Ruta N, PAHO, and DND*i*.

An international workshop with the participation of 10 experienced PCR CL laboratories from 7 Latin American countries [Argentina, Brazil (2), Colombia (3), Costa Rica, Mexico, Panama, and Peru] was carried out in December 2016. The main objective of the workshop was to compare the performance of molecular assays for the detection and quantification of different *Leishmania* species in order to establish a standardized multiplex real time PCR protocol by the absolute quantification of the parasite load and normalization by the human DNA amount, obtained from cutaneous lesion samples.

During the activities, a silica-column based protocol for DNA extraction from skin lesion samples, containing an external quality control, was standardized. In addition, the performance of three molecular targets for Leishmania was compared: SSUrDNA, kDNA, and HSP70.

Preliminary results with reference strains of the most prevalent *Leishmania* species indicated a reportable range varying from 10^6 to 5 Par. Eq./mL for the SSUrDNA and kDNA targets and from 10^6 to 50 Par. Eq./mL for HSP70. For the human RNAse P gene, a linearity from 10 to 10^{-3}ng/mL of human DNA was achieved in multiplex with *Leishmania* targets, indicating a wide range to be used to quantify the parasite and normalize data by human DNA. In patients' samples presenting high, moderate and low parasitism, all targets could be detected and parasite load estimated following this methodology. In contrast, during the evaluation of analytical specificity with reference samples from other tripanosomatids, such as *Trypanosoma cruzi, T. rangeli*, *Chritidia fasciculata,* and *Herpetomonas muscarum*, HSP70 and SSUrDNA target presented the highest specificity for the detection of *Leishmania* species.

The next step will be the standardization and clinical validation of the consensus methodology defined during this workshop. For this, the DNA extracted from ulcer samples obtained from CL patients attending health facilities in the participating countries of this initiative will be employed.

The workshop was hosted by Unidad de Biología Molecular y Computacional PECET - Programa de Estudio y Control de Enfermedades Tropicales, SIU-Sede de Investigación Universitaria - Universidad de Antioquia, Medellín, Colombia.

All scientific and technical activities were coordinated by the Laboratório de Pesquisas em Leishmanioses and Laboratório de Biologia Molecular e Doenças Endêmicas, Fundação Oswaldo Cruz, Brazil.

PCR Workshop at the University of Antioquia, Colombia, was attended by 10 international laboratories

References

[1] World Health Organization. Control of the leishmaniases. World Health OrganTech Rep Ser. 2010; (949):xii-xiii, 1-186, back cover.

[2] Reithinger R, Dujardin JC, Louzir H, Pirmez C, Alexander B, Brooker S. Cutaneous leishmaniasis. Lancet Infect Dis. 2007 Sep;7(9):581-96.

MARGRIET DEN BOER
Completed her PharmD in the Netherlands and obtained a Masters Degree in Public Health in Developing Countries at the London School of Tropical Medicine. The last 15 years she worked with Médecins Sans Frontières (MSF) and the World Health Organization (WHO), in a combination of activities related to leishmaniasis and pharmaceutical matters, including access to drugs

Could you give us a brief update on the panorama of access for leishmaniasis drugs?

M.B. Firstly, AmBisome® manufactured by Gilead, as you know there is now a donation program. That is in itself a good solution. The only concern is, of course, that it might be a temporary solution, because we don't have an agreement with Gilead that this is going to be an eternal possibility for us. Are they going to extend the agreement or not? And so far, they will.

The discussion of MSF was always to focus and enforce production at a cheap price. But, we are not there yet with AmBisome® and Amphotericin B, and we have been looking around for other producers that could maybe make a generic, but this is very difficult. We have been working with Cipla for a long time, they developed a generic, but it's quite expensive, and they say that they can't make it any cheaper, because the raw materials are expensive, the manufacturing process is expensive. All the producers that have made generics for AmBisome®, in fact, they are not true generics: they are cheaper, but their safety profiles seem to be not as good, so this means that it's an entirely different formula, that we are also not sure how effective it is going to be really, in visceral leishmaniasis. This is a concern, because the governments of many endemic countries don't have the capacity to look at the similarities between AmBisome® and these generic forms, and this has already led to some problems.

Secondly, miltefosine that it is not under patent right now and could be produced by another manufacturer. It seemed a promising drug in the beginning: it was held as the new first line drug for Asia, but it's not anymore, it's now a very marginalized drug in Asia, only for a few cases, second line treatments. Also in Africa the market is very small so far, and there is a market in South-America but it's not what we thought it would be.

Now there are the antimonials. Meglumine antimoniate is produced by Sanofi Aventis, it's a huge manufacturer, and there is some kind of agreement with WHO, it's produced, it's sustainable and low price. In most of the countries where sodium stibogluconate is used, we use a different product from a small Indian manufacturer, that is committed so far to produce it. They marketed it for a price that is a good price for them, and a good price for us, but here also there is no real, true guarantee for sustainability.

In fact, for leishmaniasis, the intellectual property is not an issue. As you said for AmBisome®, even if the patent has expired, we are not able to produce a generic drug for a cheap price.

M.B. Exactly, because it is complex to produce AmBisome®. I think Gilead feels secure, even though the patent has expired. I know some Indian manufacturers that have tried to register their products in the European Union market and they got some requirements, bioavailability standards for example. It is very expensive, so they assume that it was not worth it. There is also the price issue. Generic manufacturers must match in production with Gilead. Only a big company can keep the price down further. That would come out far too expensive to generic manufacturers. So that's true, the patent is not really an issue.

There are a lot of challenges to face, how can we improve this situation for the future?

M.B. I think there must be a highly individualized solution for each manufacturer, based on negotiation, and we must try to explore what the manufacturer wants, and then to try to get some kind of legal agreement that we can call on. But it depends if we can get a manufacturer to sign such an agreement. Of course, there must be something in it for them, and that is a difficulty. First the world market, we don't want to pay a lot of money for the drug, then they have to keep up the production to very high international quality standards. So, what's interesting in it for them? Not much. The only thing that could be interesting for them is their public image. But how much is a small manufacturer in India going to care about their public image in the world.

Maybe try to do capacity building in endemic countries, to solve their problems? Try to engage governments as well?

M.B. Of course, it would be ideal if these countries could produce their own drugs, but this is a problem, because in the country the market is even smaller, the market is only significant if you combine all the countries together. Also, to transfer production is an expensive process in itself. If you want the drugs to be produced by another manufacturer, then you would have to invest quite a lot. And it also takes a long time. Maybe all these drugs need to be adopted by the big pharma, as some kind of a social branch that they all have, that is there to meet a social responsibility criteria. And then they can be held accountable by the international public to do it. This is a solution that seems to be working quite well. Sanofi for example, they're making antimonials, and they have the sleeping sickness drugs that they are donating, Merck have worked with the ivermectin donation program, there's all these other helminthiases drugs that are being donated by the big pharma, there is AmBisome® by Gilead. On the other hand, I think that any agreement should have something to at least make the life of the manufacturers as easy as possible. So, for example, put forth that they know exactly how much they need to produce in a year, that they know that there'll be a guarantee of purchase, for at least a couple of years, that they can count on. That would really help them to help us.

Based on a Skype Interview conducted in July 2015 and updated in March 2017

Team of Synthetic Organic Chemistry Laboratory in Unicamp, Brazil, works with lead optimization in LOLA project

IN VITRO & *IN VIVO* MODELS AND CHALLENGES IN THE DISCOVERY OF NEW DRUGS FOR LEISHMANIASIS IN LATIN AMERICA

JADEL MÜLLER KRATZ
Lead Optimization Latin America Coordinator
DND*i* Latin America

Despite the efforts and investment made in the research and development of new drugs for leishmaniasis in recent years, pharmacological treatment still remains limited to a few alternatives. Drugs currently available in the market have many limitations (closely related to the physicochemical properties of their active molecules), such as high toxicity, low tolerability, parenteral administration, long duration of treatment and high cost. Thus, the pursuit of effective, safe and easily accessible treatments remains widely necessary.

It is essential that the cumulative knowledge about the parasites and the pathology of diseases be as large as possible to provide the support and tools needed by researchers. This knowledge enables the construction and a continuous update of *in vitro* and *in vivo* models that mimic infection conditions in a relatively controlled environment. These models allow the design, evaluation, and optimization of molecules that are selective enough against the parasites and have drug-like properties, that is, are able to reach the reservoirs of the parasites in the body (for example, the phagolysosomes of the macrophages in the case of *Leishmania* amastigotes).

The most commonly used strategy for the screening of *in vitro* molecules is based on the identification of leishmanicidal compounds. For this, models that can be categorized according to the stage of the parasite are used – promastigotes, axenic amastigotes or intracellular amastigotes. There are currently many protocols described in the literature, ranging from classical models based on parasite counts via microscopy to versions adapted for High Content Screening (HCS). Models based on intracellular forms have been considered the gold standard, since the amastigotes found within vacuoles in macrophages are the clinically relevant forms of the parasite. Although it is important to evaluate compounds against the different forms of parasites, screening assays based solely on promastigotes or axenic amastigotes tend to generate a higher number of false-positive results. On the other hand, a novel cytocidal-only axenic amastigotes assay has been recently developed and showed much improved translation to the intracellular assay.[1]

Animal models represent a crucial stage in the development of new molecules. Experimental models using mice and hamsters are the most common; and the latter remains relevant since the course of infection in this specie resembles that of humans, and the evaluation of parasitemia reduction in various organs (such as liver, spleen and bone marrow) is likely to be beneficial from the point of view of clinical data translation. It is important to emphasize that, although essential to the development process of antileishmanial drugs, the *in vitro* and *in vivo* models have a number of serious limitations. The use of laboratory adapted strains reduces parasite variability when compared to that found in humans, and animal models are unable to replicate the range of parasite expressions, immune response and clinical manifestations. In addition, the variability of the conditions used in different laboratories also prevents the direct comparison of results.

Despite such limitations, researchers from all over the world have successfully used *in vitro* and *in vivo* models of cutaneous and visceral leishmaniasis in a variety of ways, from host-parasite interaction studies, investigation of mechanism of action and molecular targets, to "retrotranslation" of clinical data.[2] Within medicinal chemistry projects, which aim to identify and optimize new molecules, these models usually integrate multilevel screening cascades. In brief, these projects include, apart from classic parasitology, the evaluation of selectivity, toxicity and pharmacokinetic properties (ADME/DMPK), aiming at the identification of the most promising candidates. First, the molecules are screened *in vitro* for leishmanicidal activity and *in vitro* ADME; the initial hits are then optimized through cycles of

analog synthesis and multiparametric screening. Lead compounds with superior properties proceed to a second stage of optimization, incorporating into the cascades *in vivo* evaluation of pharmacokinetic and efficacy, until a promising pre-clinical candidate is identified. Before clinical trials can be carried out, this candidate must also undergo formulation development and GLP regulatory toxicity studies.

In addition to the difficulties related to the disease itself and its models, there are specific hurdles in Latin America that make the discovery and development of new drugs for leishmaniasis difficult. First, investment in research is small when compared to other areas, such as in the field of chronic diseases. Furthermore, the difficulty in obtaining consumables, the lack of multi-centric and structured efforts, and the absence of harmonization of assays carried out by different research groups generate a gap in the development chain. Many promising results are generated in screening from several sources, but promising hits usually do not progress to leads with optimized profiles. In Brazil, only 4% of clinical trials performed to date have focused on neglected diseases, a likely reflection of the lack of long term investment and drug development capabilities.

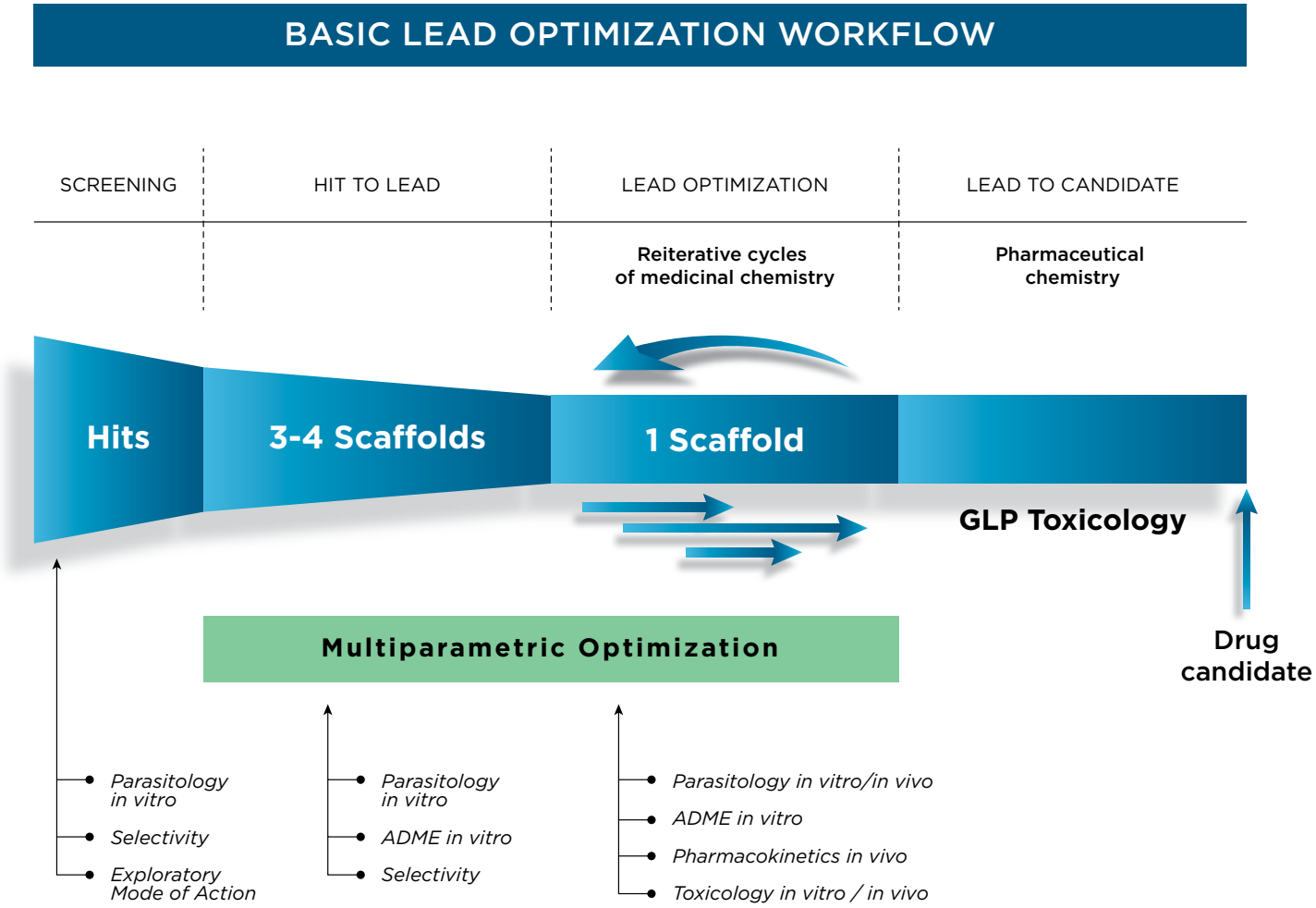
Improvement in this scenario certainly requires the formation of local expertise and joint effort of professionals through integrated research strategies. In addition, the establishment of an industrial policy for the regional pharmaceutical sector, involving the creation of public-private initiatives, and continuous availability of resources should provide support for the progress against neglected tropical diseases. In this way, DND*i* has proposed and disseminated target candidate profiles (TCP) to assist in the identification of new compounds with greater probability of success, and has also structured collaborative work forces for discovery and optimization of compounds (Lead Optimization Latin America Consortium) to boost capacities in endemic areas.

In summary, there have been relevant advances in this field and a great deal of engagement of research groups, allowing the identification of new active, selective and bioavailable compounds, which are now progressing into the development pipeline. However, the path to be followed by a new chemical entity from discovery to registration is long and costly, and the attrition rate is relentless. To maximize the chances of access to new drugs by neglected patients it is essential that all individuals involved in leishmaniasis research remain focused on further understanding the disease, and also on improving preclinical models and research strategies, not forgetting the importance of valuing science and promoting a favorable environment for research in Latin America.

References

[1] Nühs, A., et al. Development and validation of a novel Leishmania donovani screening cascade for high-throughput screening using a novel axenic assay with high predictivity of leishmanicidal intracellular activity. PLoS Negl. Trop. Dis., 9, e0004094, 2015.

[2] Khare, S., et al. Proteasome inhibition for treatment of leishmaniasis, Chagas disease and sleeping sickness. Nature, 537, 229-233, 2016.



Moacir Zini in his mechanical workshop, Ipiranga do Norte, Mato Grosso, Brazil

PATIENTS WITH NEGLECTED DISEASES' ASSOCIATION: AN OPPORTUNITY FOR LEISHMANIASIS

MADY BARBEITAS
redeLEISH Coordinator
DND*i* Latin America

THE CONTEXT

On June 6th 2016, the workshop "Interfaces between Social Movements and NGOs in Confronting Neglected Tropical Diseases: an Analysis of its Limitations and Potentialities" was held in Rio de Janeiro. Bringing together representatives of many entities and social movements, this workshop was part of the program organized by DND*i* Latin America during the Clinical Research Platforms' annual meeting on Chagas Disease and Leishmaniasis (Chagas Platform and redeLEISH), which preceded the DND*i* Innovation & Access – Partners' Meeting 2016.

The workshop aimed at promoting an initial opportunity for the discussion and exchange of experiences between several entities involved in specific issues of public health and neglected diseases. There was for example, a large attendance of associations of people affected by Chagas Disease, Leprosy and Hepatitis C in different regions of Brazil and abroad. Considering that these diseases are categorized as "neglected" as they face common barriers such as lack of research, access and innovation, we evaluated the need to promote an integrated agenda to enhance knowledge and mobilization to face the diseases, and sought to identify strategies to ensure sustainability for this interface. Furthermore, this event gave us the opportunity to bring together patients with leishmaniasis (cutaneous and visceral), amongst them, Mr. Moacir Zini and his wife Talita.

BRIEF HISTORY OF SOCIAL MOVEMENTS THAT CHANGED THE COURSE OF PUBLIC POLICIES

The importance of this workshop was seen in view of the current Brazilian political-institutional scenario and also in the context of an observed disengagement of civil society. Historically, various associations have mirrored the HIV/AIDS movement, which generated an agenda based on human rights and achieved a significant impact in terms of public policies. HIV/AIDS was an era-defining movement, with patients´ associations directly influencing the course of the disease and its therapeutic progress. Some spectacular lawsuits have set precedents in the Brazilian law to grant free and universal access to antiretroviral drugs. Less widespread than HIV/AIDS associations, yet however no less efficacious, associations of patients with rare diseases in Europe (such as Eurodis and others) contribute to drug innovation by interacting directly with industry, policy makers and scientific community. One of their feats was the lobbying for the revision of the bioethics law, resulting in the approval of the amendment known as "savior baby" or "savior sibling", which allows the genesis of a fetus for therapeutic purposes.[1]

> *"I only fear the day when I am not going to be able to take medication any more. I fear the day when you have the medication in front of you and you say: if I take it, I am going to die, then, I have to carry on without taking it."*
>
> *Moacir Zini*

THE PROBLEMATIC OF LEISHMANIASIS

Leishmaniasis patients rely entirely on physicians and health professionals for correct guidance on treatment, replicating their conduct and practices. In fact, physicians and specialists are the professionals truly responsible for the generation and diffusion of knowledge on leishmaniasis, being, therefore, the only spokesmen for this condition. Consequently, actions for tackling the disease only address its biomedical aspects (search for alternative treatment schemes, which drug to prescribe for a given clinical framework or for a specific *Leishmania* species), and organized socio-political actions are rarely observed. This can be explained by the fact that the disease is closely related to low levels of education in the population as well as by epidemiological factor (low concentration of cases per municipality, cases spread in a vast geographical area). Another aggravating factor is

that leishmaniasis has a weak media appeal due to the misconception that it is not a deadly disease.

THE EXPERIENCE OF MR. ZINI

Moacir Zini is a patient with cutaneous leishmaniasis in its most aggressive form, the so called diffuse leishmaniasis. Contrary to most observed cases, Moacir has lived with the disease for over 26 years, as he explains: "*Many people ask me why I have leishmaniasis for 26 years, there are no reports of such cases lasting that long. If you get visceral leishmaniasis, either you get cured or it kills you. So, it lasts something like thirty, or forty days and then it is all over. You even forget about it. Cutaneous leishmaniasis usually goes on for one or two years before you get cured. However, the diffuse form, which is the type I have, it is very slow... The first doctor who made the diagnosis joked with me: 'It is good that it is leish because if it was malaria it could be worse'. But I am not so sure...*".

Moacir went through innumerous treatments and suffered greatly from the toxicity caused by the medicines. He suffered a heart attack, had high blood pressure and left kidney failure. I asked him if he was not afraid of dying because of the drugs: "*I only fear the day when I am not going to be able to take medication any more. I fear the day when you have the medication in front of you and you say: if I take it, I am going to die, then, I have to carry on without taking it.*" The experience of each patient is unique, in the sense that he / she is the only one who knows what it means to live with the disease. Their needs are dictated by the proximity with the disease and this aspect is not always considered by the medical discourse.[2] It is therefore important that patients come together to demand their rights for research and better drugs. Mobilization of people is what makes political decisions come true and be carried forward as country policies.

> *"For the future, we must focus on projects with the municipalities, bring knowledge about the disease, so to enable a rapid and accurate diagnosis. It should go beyond the big cities."*
>
> *Moacir Zini*

To continue the movement initiated during the workshop, a second meeting was held in August 2016 in the context of the Annual Meetings of Applied Research on Chagas Disease and Leishmaniasis at the Brazilian Society of Tropical Medicine Congress, which resulted in the creation of the Brazilian Social Forum for Confronting Infectious and Neglected Diseases. Moacir told me about his experience at this second meeting: "*It was very good; I even learned more about the disease, and I had the opportunity to share my experience with others, but we need support, we need to invite more people to attend these meetings and try to make something concrete happen. We must show that the association exists and we want to take part in the decision making. Talita and I even tried to approach the City Hall of Ipiranga do Norte (a city in the state of Mato Grosso), but since there is only me, it was not easy*".

I asked Moacir: How do you see the creation of this forum as an opportunity to assist patients with leishmaniasis? "*The Association of leishmaniasis patients would be quite weak on its own because there are few people who would agree with public exposure. Hence, the importance of working together. For the future, we must focus on projects with the municipalities, bring knowledge about the disease, as to enable a rapid and accurate diagnosis. It should go beyond the big cities*".

The next meeting of the Forum will be in August 2017, in Cuiabá, and we hope to have more voices for leishmaniasis. The movement is still gaining momentum, but it is undoubtedly a great opportunity to fight against this disease that, until recently, had only one association in Brazil, and it was for canine leishmaniasis.

References

[1] S. Dalgalarrondo, « Quelle place pour les associations de malades dans le processus d'innovation médicamenteuse? Le cas des maladies rares », Revue française des affaires sociales 2007/3 (nº 3-4), p. 171-191.

[2] V. Rabeharisoa, M. Callon, « L'engagement des associations de malades dans la recherche», Revue internationale des sciences sociales 2002/1 (nº 171), p. 65-73.

Meeting of the patients Forum at the Brazilian Congress of Tropical Medicine, Maceio, Brazil

redeLEISH webforum

Virtual network of investigators in cutaneous leishmaniasis.

SIGN IN:

http://platforms.dndi.org/redeleish

What is it?

The redeLEISH webforum is an online platform easy to access which allows interaction between participants. Designed for Latin America countries, the main languages are Spanish and Portuguese.

With the webforum it is possible:

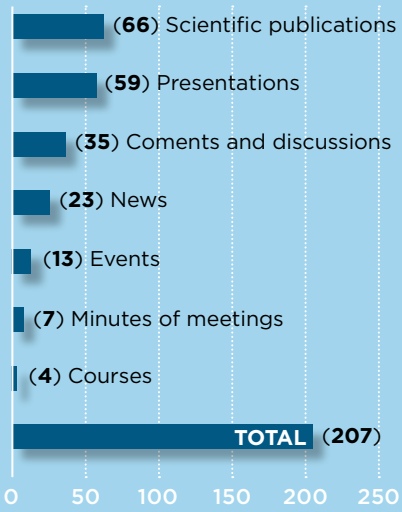
- To exchange information / discuss treatment options, diagnostics, clinical trials, R&D, drug access and disease control.
- To share the latest scientific articles.
- To circulate and add comments about relevant news.
- To disseminate courses and events.
- To access articles and the annual meeting presentations in the library.
- To create surveys.

How?

- All in your email inbox!
- Send an email to redeleish.coordenacao@dndi.org or sign in directly in the website http://platforms.dndi.org/redeleish

POSTS ON THE WEBFORUM

Until February 2017



ACKNOWLEDGMENTS AND CREDITS:

redeLEISH has the support of the Brazilian Development Bank (BNDES), Ruta N, Secretariat of Science, Technology and Health Supplies of the Brazilian Ministry of Health (SCTIE / DECIT) under the Cooperation and Technical Assistance Agreement signed between Ministry of Health / Secretariat of Science, Technology and Strategic Supplies (SCTIE), FIOCRUZ and DND*i*.

Editorial board:
Byron Arana, Joelle Rode, Mady Barbeitas & Marina Boni

Text editing:
Mady Barbeitas

Production:
Julia Alapenha

Graphic design:
Charles Savry & Bruno Silva

Translation and text revision:
Jayme Fernandes, Joelle Rode, Júlia Alapenha, Mady Barbeitas, Marcela Dobarro, María Raquel Barrera & Marina Boni

Photo credits:
Joelle Rode (DND*i*): P3, Marina Boni (DND*i*): P4, Photo courtesy (PECET): P6, Paul Koovitz (Unicamp): P8, Mariana Abdalla (DND*i*): P10 & Laura Acebal (DND*i*): P11

DNDi LATIN AMERICA
Drugs for Neglected Diseases *initiative*

DND*i* Latin America
Rua Santa Heloisa 5
Jardim Botânico
Rio de Janeiro – RJ - 22460-080
Brasil - Tel: +55 21 2529 0400
www.dndial.org

DND*i* Headquarters
15 Chemin Louis-Dunant
1202 Geneva - Switzerland
Tel: +41 22 906 9230
Fax: +41 22 906 9231
www.dndi.org

SUPPORT:

ruta n MEDELLIN
Ministério da Saúde FIOCRUZ Fundação Oswaldo Cruz
BNDES
Ministério da Saúde
BRASIL GOVERNO FEDERAL