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REVIEW

Recent Development of Visceral Leishmaniasis Treatments: Successes, Pitfalls, and Perspectives

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SUMMARY Research in visceral leishmaniasis in the last decade has been focused on how better to use the existing medicines as monotherapy or in combination. Systematic research by geographical regions has shown that a universal treatment is far from today's reality. Substantial progress has been made in the elimination of kala-azar in South Asia, with a clear strategy on first- and second-line therapy options of single-dose liposomal amphotericin B and a combination of paromomycin and miltefosine, respectively, among other interventions. In Eastern Africa, sodium stibogluconate (SSG) and paromomycin in combination offer an advantage compared to the previous SSG monotherapy, although not exempted of limitations, as this therapy requires 17 days of painful double injections and bears the risk of SSG-related cardiotoxicity. In this region, attempts to improve the combination therapy have been unsuccessful. However, pharmacokinetic studies have led to a better understanding of underlying mechanisms, like the underexposure of children to miltefosine treatment, and an improved regimen using an allometric dosage. Given this global scenario of progress and pitfalls, we here review what steps need to be taken with existing medicines and highlight the urgent need for oral drugs. Furthermore, it should be noted that six candidates belonging to five new chemical classes are reaching phase I, ensuring an optimistic near future.

KEYWORDS visceral leishmaniasis, new chemical entities, treatment

INTRODUCTION

Leishmaniasis is a complex vector-borne disease, with more than 20 causative species of *Leishmania* protozoa resulting in diverse disease manifestations, ranging from localized skin ulcers (cutaneous leishmaniasis) to systemic disease that can be fatal

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if untreated (visceral leishmaniasis [VL]) (1). Leishmaniasis has strong links with poverty, due to poor housing conditions and deteriorated environmental sanitation, and with low income, gender imbalance, wars and displacements, immunosuppression, and poor nutrition, among other determinants (2).

VL, also known as kala-azar, is mostly caused by *Leishmania donovani* in Asia and Eastern Africa and by *Leishmania infantum* in Latin America, Central Asia, and the Mediterranean region. The parasites are transmitted through the bite of the female phlebotomine sand fly. In the human host, they are obligate intracellular parasites of the reticuloendothelial system and survive and multiply in various macrophage populations. A proportion of infected individuals will present with symptoms which evolve insidiously, with splenomegaly, irregular fever, anemia or pancytopenia, weight loss, and weakness occurring progressively over a period of weeks or even months. Currently, there are no validated biomarkers to predict which immunocompetent individuals are at risk of developing VL after infection.

The natural history of VL is complex, as various elements fuel transmission, often in the context of poverty, with sand fly vectors and human and animal reservoirs as key elements in the transmission chain. However, there are other leishmaniasis forms that may also play a role in transmission, such as post-kala-azar dermal leishmaniasis (PKDL), HIV-VL coinfection, and potentially, asymptomatic carriers. These topics deserve special attention from the epidemiological and clinical points of view, have been reviewed recently, and are therefore beyond the scope of this article (3–7). In Asia and Africa, VL is anthroponotic, although this notion remains controversial for the latter, while in Latin America and the Mediterranean region, VL is a zoonotic disease, with the dog as the main reservoir. In 1990, the World Health Organization (WHO) estimated the worldwide incidence of VL to be 500,000 new cases annually, but these figures were subsequently updated to an average of 58,221 new VL cases reported annually based on a 5-year reporting period (2004 to 2008): estimates ranged from 202,100 to 391,400, adjusting for underreporting (8). Six countries accounted for 90% of all cases: India, Bangladesh, Sudan, South Sudan, Ethiopia, and Brazil. A more recent report by WHO on the 14 high-burden countries ($>$100 cases/year) shows a decrease in overall cases reported, down to 30,758 new cases in 2014, with underreporting estimated to be from 1.2- to 4-fold (9). This reduction in the number of VL cases is mainly due to a sharp decrease in reported cases in South Asia, dropping from approximately 50,000 reported cases in 2007 to 6,746 cases in 2016, and can be attributed to a number of factors, including the successful elimination campaign, the naturally fluctuating trend of incidence, and improvement in the living conditions of the local population. Currently, the region with the highest burden worldwide is Eastern Africa, with most cases observed in Ethiopia, Kenya, Somalia, Sudan, South Sudan, and Uganda. Somalia has replaced Bangladesh in the list of the top six countries affected (9).

The Kala-azar Elimination program (KAEP) is based on five pillars: early case detection for prompt diagnosis and treatment, surveillance, vector control, social mobilization, and operational research. In this paper, we focus on the first pillar, rapid access to diagnosis and treatment, especially in anthroponotic foci, for case management and to interrupt the transmission cycle. In routine care, simple rapid diagnostic tests (RDTs) are ideal, as they can be deployed in low-resource settings. Serological tests based on the recombinant kinesin of 39 KDa (rK39) are available for screening of suspected VL cases. They have high sensitivity and specificity in the Indian Subcontinent but lower sensitivity in Eastern Africa and Latin America (10, 11). Furthermore, serological tests cannot be used for test of cure or diagnosis of relapse cases, as antibody levels remain detectable for years after a VL episode. In clinical research, a confirmatory parasitological diagnosis is needed, as well as a reliable test of cure, to objectively assess treatment efficacy. The gold standard for VL diagnosis and test of cure is direct parasite visualization by microscopy of tissue aspirate samples (spleen, bone marrow, or lymph node). Sensitivity differs according to the biological material used, spleen aspirate being the most sensitive and lymph node aspirate the least sensitive. However, the tissue aspiration is invasive and methods are difficult to harmonize. Molecular tests, such as

PCR on blood, are very sensitive but require trained personnel and laboratory resources that may not be practical in the field, and this method is still not validated to replace actual parasitological visualization. Urine antigen detection tests are highly specific but currently not sensitive enough to be widely used, although they would be ideal as a noninvasive test of cure (12).

The Th2 response suggests the immune response in VL is inefficient in controlling *Leishmania* infection, evolving to the death of the patient if untreated. Both treatment and immune response contribute to the success of the cure of the patient. The presence of parasites at end of treatment (EoT) can imply a nonsterile cure, while the balance between immune system activity and the few remaining parasites promotes survival of the patient with no impairment. Alternatively, if parasites remain at somewhat higher levels, this may lead to a slow but progressive increase in parasite numbers, leading to relapse within months of treatment.

The panoply of drugs available for VL is limited to antimonials (sodium stibogluconate [SSG] and meglumine antimoniate [MA]), paromomycin (PM), oral miltefosine (MF), and amphotericin B, the latter in two formulations, the free deoxycholate form and lipid formulations, with liposomal amphotericin B (LAB) among the latest. VL treatments have made much progress over the last 15 years. Treatment options have moved from a reliance on antimonial monotherapy to the development of new treatments, including different lipidic formulations of amphotericin B, the oral drug MF, and injectable PM. In past years, clinical data have shown that the same drug and dosing regimen may not have the same efficacy depending on the geographical area of use. In addition, particularities of patient populations, such as genetic and anthropometric characteristics, immune status, and also the social and epidemiological context, may influence outcome. Despite the improvements achieved in the last decade in the development of new drugs, the need for an innovative therapy that is safe and can be deployed in remote areas where VL occurs, ideally with pangeographical efficacy, is badly needed. Moreover, the characteristics of each medicine may determine which one should be chosen at the individual or collective level of use, in primary health care systems or in referral hospitals, in routine health care or in an elimination program, and if the later, in the attack or maintenance phases, and finally, which medicines to use in veterinary health versus human health to protect against resistance.

Clinical development in the past decade, which is central to DND*i*'s agenda, has focused on improving VL treatment with the existing medicines as monotherapy or in combination to improve their efficacy and safety and from long-duration regimens to short courses or even a single dose. Ultimately, this research has led to the adoption of new treatments, such as SSG-PM in Africa and LAB or PM-MF in Asia, under WHO and national guidelines. By 2020 to 2021, it is expected that all studies to assess the existing medicines for treating VL (and also HIV-VL and PKDL) will be completed (Fig. 1). Unfortunately, the progress made in clinical research is not always translated into implementation in the short term, with multifactorial challenges in the process to achieving changes in national guidelines.

Finally, although it is known that the outcome of VL is mediated by the immune response and that drugs with immunomodulatory effects should contribute to the recovery of the patient, clinical trials to understand the contribution of immunomodulation to cure are still in the future. In the meantime, a number of orally administered drugs are progressing to clinical development in 2018, aiming to innovate with efficacious oral, short-course, and safe treatments tailored for deployment in remote areas. This paper aims to summarize all efforts made in the past decade to improve VL treatment according to the various geographical contexts, followed by an analysis of the remaining needs and perspectives for future research and development.

SUCCESSES AND PITFALLS

Anthroponotic Visceral Leishmaniasis

Anthroponotic VL is distributed in South Asia and Eastern Africa, accounting for more than 95% of all VL cases worldwide. As the infected human is the reservoir, early

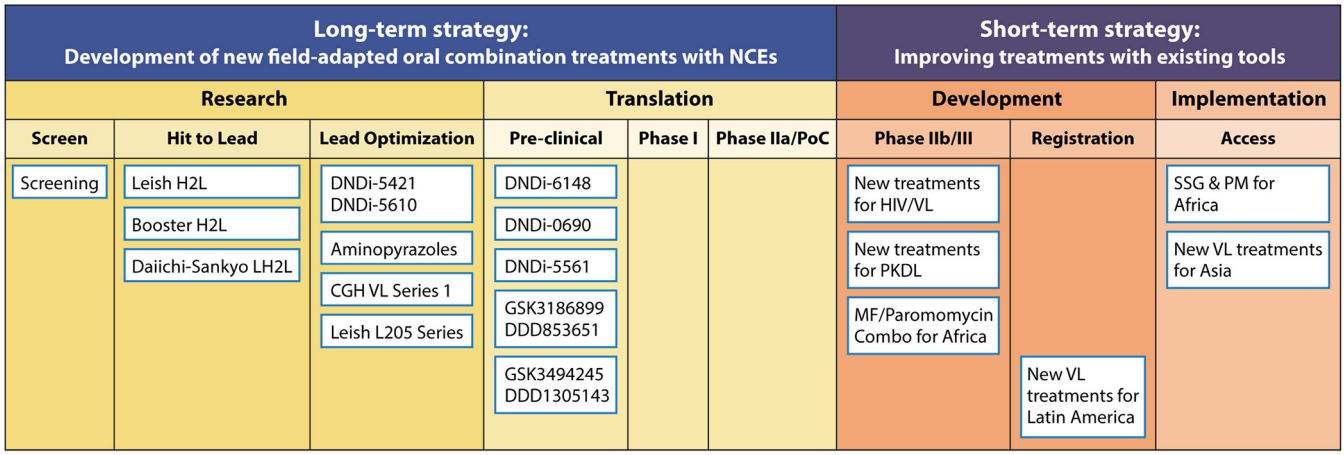

FIG 1 DND*i* leishmaniasis portfolio from discovery to implementation.

treatment aims not only to cure the patient but also to contribute to decreasing the transmission cycle.

South Asia region. VL in South Asia is a disease typically linked with poverty, afflicting poor populations, 68% of whom live in mud houses with straw roofs and in proximity to cattle and 75% of whom have a *per capita* income of <$1 a day (2). It affects the most vulnerable, and certain lower social groups are more affected than others. The disease is concentrated in one geographical area, including four Indian States (Bihar, West Bengal, Jharkhand, and Uttar Pradesh), the Terai region of Nepal bordering Bihar in India, and most of the territory of Bangladesh, although it is more prevalent in the north central provinces.

The history of drug development for VL is intimately linked to India. VL patients have been treated extensively since the 1920s with pentavalent antimonials in two possible formulations, SSG or MA (Glucantime). The former was used predominantly in the Indian subcontinent and Eastern Africa, and the latter in the Mediterranean region and Latin America. Both have a similar efficacy and safety profile and are given intramuscularly (i.m.) (or intravenously [i.v.]) for 28 days at a daily dose of 20 mg/kg of body weight when used as monotherapy. The large volume to be injected daily and the drug itself cause great pain at the injection site, which threatens its use. Elevation of pancreatic and liver enzymes may require treatment interruption, but of more serious concern is the cardiotoxicity (prolonged QTc interval, arrhythmias, ventricular fibrillation, etc.) that may progress to sudden death, in particular in older patients. The long treatment duration and injection pain led to its misuse in India, where the treatment was often given only for 2 weeks, until the fever disappeared. In the 1980s, reports on ineffectiveness in India led to an increase in treatment duration of up to 4 weeks with the 20-mg/kg/day regimen (13). The longer treatment duration initially improved treatment effectiveness, but soon after, resistance levels rose in Bihar, leading to a mere 40% cure rate on the left bank of the Ganges, compared with an efficacy of 86% in Uttar Pradesh (14), linked to different levels of parasite sensitivity. SSG resistance ultimately spread to surrounding countries (15).

At the beginning of the 21st century, the only alternative for rescuing patients with no response to antimonial treatment or for treating relapses was the polyene antibiotic amphotericin B deoxycholate, which was very effective but required prolonged hospitalizations for 15 to 20 i.v. infusions (0.75 to 1.0 mg/kg/day by infusion, daily or on alternate days for 15 to 20 doses). Monitoring the severe and common nephrotoxicity effects and hypokalemia is mandatory (16). Due to its poor safety profile, this drug was restricted to teaching and district hospitals.

In 2002, MF, an oral alkyl phospholipid, was incorporated into the limited panoply of anti-*Leishmania* drugs, opening an optimistic new landscape that mobilized policy

makers in the Indian subcontinent. This was a major development after the frustration of 3 decades of increasing kala-azar incidence following the malaria campaigns of the 1970s, when VL was almost eliminated as a side effect of using DDT for mosquito control. Indeed, in 2005, the Ministers of Health of Bangladesh, India, and Nepal signed a Memorandum of Understanding to eliminate VL (defined as $<$1 case per 10,000 people at the upazilla/block/district level, equivalent administrative levels according to the cited countries) by 2015. Five pillars supported the KAEP: case management based on diagnosis with the rK39 rapid diagnostic test (RDT) and prompt treatment with MF, vector control, surveillance, social mobilization, and operational research (17). MF was included in 2005 in the National Guidelines of India, Nepal, and Bangladesh as a first-line treatment for VL following a phase III trial in India that showed a final cure rate of 94% (18). Due to its long treatment course (28 days), the high cost of the treatment ($65 to $150), and poor medical practices in the private sector, with the drug available over the counter, compliance was a challenge (19), limiting proper roll out. Moreover, the efficacy of MF declined to 90% within the last decade of use in South Asia, with a 2-fold-increased risk of relapse, from 3% to 6.8% (20). Rijal et al. (21) showed that in Nepal, treatment failure rates due to relapse were 10.8% and 20% at the 6- and the 12-month follow-up, respectively, where relapse was more common in children $<$12 years old and was associated with an $\sim$30% lower MF concentration at the EoT compared to that in adults (22). Initial cases and relapses were confirmed to be due to the same parasite through fingerprinting (no reinfection), and no significant change in parasite susceptibility was identified pre- versus posttreatment (21). However, more recently, parasites in two patients in India have been characterized with resistance to MF (23).

In parallel, PM, an aminoglycoside used in the treatment of intestinal amoebiasis and with known activity in leishmaniasis, was rescued from oblivion 40 years after its activity against *Leishmania* had been shown in mice (24). A phase III clinical trial in India demonstrated that i.m. PM at a dose of 15 mg/kg for 21 days provided a final cure rate of 94.6% (25), but with the rapid introduction of MF and, later, LAB, this regimen was never implemented in Asia. However, PM has proven to have a major impact when used in combination with SSG on African VL (see below), but as with MF, there are *in vitro* indications that resistance may easily develop when used in monotherapy (26).

Similarly, LAB, used in fungal diseases, had shown activity against *L. donovani* in two animal models about 20 years ago (27). The antifungal is intercalated into a liposomal membrane consisting of phosphatidylcholine, cholesterol, and other phospholipids, forming small unilamellar bilayer liposomes. LAB is rapidly phagocytized by the parasite-infected macrophages in target tissues like the liver and spleen, where it binds to parasitic ergosterol, a major component of the parasite membrane. This results in disruption of the parasite membrane, thus killing the parasite directly in the macrophage, and in almost no amphotericin B in the plasma, the free fraction that causes renal damage (28). Various studies using different LAB regimens showed high efficacy and very low toxicity, minimizing the potential for nephrotoxic side effects. In a randomized dose-finding study comparing 6-, 10-, and 14-mg/kg total doses of LAB, all three regimens achieved an efficacy of $>$95% (29). Furthermore, a study aiming to define the lowest but most efficacious single dose showed that one injection of 7.5 mg/kg yielded a 90% cure rate (30), whereas another study proved that a single dose of 3.75 mg/kg reached a similar efficacy of 89% (31). A major limitation of this medicine was its high price, making it unaffordable for large-scale implementation (in India it used to cost approximately $1,600 per treatment course). In order to harmonize the various regimens and start negotiations with Gilead Sciences for reducing the prohibitive cost of LAB, WHO convened a consultative expert meeting in 2005 to provide guidelines on its use according to geographical areas (32). Based on this, a price reduction to $18/vial ($160 to $200/course depending on body weight) was achieved in 2007, and simultaneously, a key publication showing that a 10-mg/kg total dose in one single infusion achieved 95.7% efficacy in India (33), bringing this drug back into the VL control toolkit. Moreover, the donation of LAB (AmBisome) by Gilead Sciences

to WHO for 5 years (2012 to 2017), and recently extended for a further 5-year period, has changed the scenario in terms of case reduction.

The kala-azar burden has been reduced rapidly by the use of MF in Bangladesh and Nepal and by single-dose LAB at the public health level, based on active case detection following this algorithm: fever of more than 14 days with splenomegaly in someone from an area of endemicity, negative for malaria, and positive leishmaniasis RDT. Treatment of such patients meeting these criteria with a 2-h infusion of LAB (after previous allergy testing) and a few hours of observation is performed before discharging the treated patient (34). The efficacy of this approach was substantiated at the subdistrict level in Bangladesh, in settings with local doctors and poor resources (35). Although patients are followed up for a period of 6 months after treatment in the clinical trials, it has been shown that relapses also occur with LAB between 6 and 12 months (0.3% and 3.7% relapsed at 6 and 12 months; mean of 9.6 months) (36).

A strong recommendation for an alternative to VL monotherapy treatment was made to combine existing drugs, with the aim of reducing treatment duration, improving safety and efficacy, increasing compliance, and avoiding the development of resistance in the long term (37). Two short combination regimens of MF-LAB and PM-MF, as well as LAB monotherapy, were evaluated in a phase III clinical trial conducted in India (2008 to 2010). All showed an excellent safety profile and efficacy of at least 97% under controlled conditions in a referral hospital (38). In order to evaluate the efficacy of these combination therapies under field conditions, two large studies were carried out to evaluate the safety and effectiveness in subdistrict (upazilla) hospitals in Bangladesh (39) and India between 2010 and 2015 (V. Goyal, R. Mahajan, K. Pandey, S. N. Singh, R. S. Singh, N. Strub-Wourgaft, F. Alves, V. N. Rabi Das, R. K. Topno, B. Sharma, M. Balasegaram, C. Bern, A. Hightower, S. Rijal, S. Ellis, T. Sunyoto, S. Burza, N. Lima, P. Das, and J. Alvar, submitted for publication), ultimately aiming to provide evidence under field conditions for policy makers. Both studies showed high-efficacy outcomes and very good safety profiles when used within the program. When the preliminary results of these studies became available in August 2014, the Indian government changed its treatment policy, so that single-dose LAB became the first option, where a cold chain is available, and PM-MF the second option, for remote areas where a cold chain cannot be ensured. The same policy was adopted by the Regional Technical Advisory Group in 2015 and later by the three countries engaged in the KAEP. Drug procurement is a recurrent problem in India, and in practical terms, PM-MF is only introduced in some referral hospitals under DND*i* influence to rescue relapsing patients treated with LAB.

As there were large numbers of patients treated in the Indian study ($n = 1{,}761$ patients), the National Vector Borne Disease Control Program (NVBDCP) requested that subjects be followed up for 12 months posttreatment to identify late relapses. Final intention-to-treat (ITT) results at the 6-month follow-up showed that effectiveness was 91.4% (89.3 to 93.1%) for single-dose LAB, 88.8% (85.1 to 91.9%) for LAB-MF, and 96.9% (95.0 to 98.2%) for PM-MF (Goyal et al., submitted). Interestingly, in the complete case analysis cure rates were 95.5% for LAB, 95.5% for LAB-MF, and 99.6% for PM-MF at 6 months (Goyal et al., submitted), whereas at the 12-month follow-up the levels of efficacy in all arms decreased to 93.7%, 91.5%, and 98.6%, respectively (V. Goyal, R. Mahajan, K. Pandey, F. Alves, N. Strub-Wourgaft, V. N. Rabi Das, C. Bern, A. Hightower, S. Rijal, T. Sunyoto, S. Burza, N. Lima, P. Das, and J. Alvar, unpublished data). Table 1 summarizes the clinical trials conducted in South Asia, including the diagnostic criteria used.

Eastern Africa region. Eastern Africa is the region that currently harbors the highest burden of VL worldwide (9). The disease affects mainly poor communities in remote rural areas. Extreme poverty, malnutrition, and displaced populations are frequently linked to large epidemics where drug access is unreliable due to weak health systems. In Eastern Africa, in the arid and semiarid areas where the population is nomadic, epidemics tend to occur when nonimmune populations move into areas of endemicity because of the extreme temperatures. In the hot season, Sudanese communities sleep

TABLE 1 Recent clinical trials on visceral leishmaniasis in South Asia

Country	Site(s)	FPFV–LPLV (mo yr)[a]	Study design	Treatment(s) studied (drug[s], dosage[s])[b,c]	Total no. of patients enrolled in study; no. per arm[b]
India	Rajendra Memorial Research Institute of Medical Sciences Patna, Chapra District hospital; Hajipur district hospital, Vaishali; 7 PHCs in Chapra and Vaishali districts	Aug 2012–Sep 2015	Open-label, prospective, nonrandomized, noncomparative, multicentric trial conducted within public health facilities in Bihar	(i) LAB 10 mg/kg in single dose; (ii) PM 11 mg/kg/day + MIL 2.5 mg/kg/day for 10 days each; (iii) LAB 5 m/kg single dose + MIL 2.5 mg/kg/day for 7 days	*n* = 1,761; (i) 891, (ii) 512, (iii) 358
Bangladesh	Community-Based Medical College Bangladesh (CBMC,B) and the Upazila Health Complexes of Trishal, Bhaluka, and Fulbaria	Jul 2010–Mar 2014	Randomized, controlled, open-label, parallel-group phase III clinical trial	(i) LAB 5 m/kg in single dose + MIL 2.5 mg/kg/day for 7 days; (ii) LAB 5 m/kg in single dose + PM 11 mg/kg/day for 10 days; (iii) PM 11 mg/kg/day + MIL 2.5 mg/kg/day for 10 days each; (iv) LAB 5 mg/kg on days 1, 3, and 5, total dose 15 mg/kg	*n* = 601; (i) 142, (ii) 159, (iii) 142, (iv) 158
India	2 sites: Kala-Azar Medical Research Center, Muzzafarpur of Banaras Hindu University, Varanasi and Rajendra Memorial Research Institute of Medical Sciences at Patna	Jun 2008–Jan 2010	Randomized, open-label, parallel-group, controlled trial based on a noninferiority design	(i) Amp B 1 mg/kg alternate days for 30 days, total dose 15 mg/kg; (ii) LAB 5 m/kg in single dose + MIL 2.5 mg/kg/day for 7 days; (iii) LAB 5 m/kg in single dose + PM 11 mg/kg/day for 10 days; (iv) PM 11 mg/kg/day + MIL 2.5 mg/kg/day for 10 days each	*n* = 634; (i) 157, (ii) 160, (iii) 158, (iv) 159
India	Institute of Medical Sciences, Banaras Hindu University, Varanasi; Kala-azar Research Centre, Brahmpura, Muzaffarpur; Balaji Utthan Sansthan, Patna, Bihar; and the Rajendra Memorial Research Institute of Medical Sciences, Patna, Bihar	Jun 2003–Nov 2004	Randomized, comparative, controlled, open-label phase III study	(i) PM 11 mg/kg/day for 21 days; (ii) Amp B infusion 1 mg/kg alternate days for 30 days, total dose 15 mg/kg	*n* = 667; (i) 502, (ii) 165
Bangladesh	Muktagacha upazila hospital (subdistrict hospital) of the district of Mymensingh	Mar 2012–Aug 2012	Open-label, nonrandomized study to assess the effectiveness, safety, and tolerability of LAB 10 mg/kg at rural public hospital	LAB 10 mg/kg	300
India	Institutes of Medical Sciences, Banaras Hindu University, Varanasi; the Kala-azar Research Center, Brahmpura, Muzaffarpur; and Balaji Utthan Sanastan, Patna	Jul 1999–Jul 2000	Randomized, open-label, controlled phase III clinical trial	(i) MIL 2.5 mg/kg/day for 28 days; (ii) Amp B 1 mg/kg alternate days for 30 days, total dose 15 mg/kg	*n* = 398; (i) 299, (ii) 99
India	Kala-Azar Medical Research Center at Muzzafarpur, affiliate to Banaras Hindu University in Varanasi	Sep 2009–Nov 2010	Open label, noncomparative	MIL 2.5 mg/kg/day for 28 days	567
Bangladesh	12 outpatient centers in regions of VL endemicity in Dhaka, Rajshahi, and Mymensingh, Bangladesh	Oct 2006–Sep 2007	Open label, single group	MIL 2.5 mg/kg/day for 28 days	*n* = 977
India	13 centers in Bihar: private centers, nursing homes, and Rajendra Memorial Research Institute of Medical Sciences		Open label, single arm to investigate feasibility of treatment of VL patients with MIL under field conditions in 13 centers in Bihar	MIL 2.5 mg/kg/day for 28 days	*n* = 1,132
Nepal	B. P. Koirala Institute of Health Sciences	Oct 2008–Apr 2011	Prospective cohort study	MIL 2.5 mg/kg/day for 28 days	*n* = 120
India	Kala-Azar Medical Research Center at Muzzafarpur, affiliate of Banaras Hindu University in Varanasi	Feb 2008–Mar 2009	Open-label prospective study	(i) LAB 10 mg/kg; (ii) Amp B 1 mg/kg alternate days for 30 days, total dose 15 mg/kg	*n* = 412; (i) 304, (ii) 108

[a]FPFV, first patient first visit; LPLV, last patient last visit.
[b]Data in different columns for different arms are indicated by small Roman numerals.
[c]LAB, liposomal amphotericin B (AmBisome); PM, paromomycin (11 mg/kg paromomycin base is equivalent to 15 mg/kg per day of paromomycin sulfate); MIL, miltefosine; Amp B, amphotericin B deoxycholate.
[d]EoT, end of treatment.
[e]95% CI, 95% confidence interval (not given in all cases); ITT, intention to treat; CC, complete case; PP, per protocol; diff, difference.
[f]CTRI (Clinical Trials Registry—India) number was not compulsory before 2007.
[g]95% CI data taken from an other source (20).

TABLE 1 (Continued)

Diagnostic criteria	Test of cure[d]	% efficacy (95% CI) at 6 mo follow-up per arm[b,e]	Reference; clinical trial no.[f]
Positive rK39 test with clinical symptoms of VL and parasitologically confirmed via bone marrow or spleen aspirate specimens in case of previously treated VL patients	Initial cure at EoT = absence of fever, clinical improvement, reduction in spleen size; final cure at 6 mo = clinical improvement with no signs or symptoms of relapse; bone marrow or spleen aspirate confirmation only in case of VL relapse	(i) ITT 91.4 (89.3–93.1), CC 95.5 (93.9–96.8); (ii) ITT 96.9 (95.0–98.2), CC 99.6 (98.6–99.9); (iii) ITT 88.8 (85.1–91.9), CC 95.5 (92.7–97.5)	Goyal et al., submitted; CTRI/2012/08/002891
Positive rK39 test and parasitologically confirmed via bone marrow or spleen aspirate specimens	Main outcome of final cure = initial cure at day 45 and absence of VL signs and symptoms during follow-up period of 6 mo; secondary outcome of initial cure = clinical improvement at day 45 (in CBMC hospital setting, initial cure was confirmed by absence of parasites in spleen/bone marrow aspirate specimens at day 15; in cases of 1+ parasite at day 15, patients were retested at day 45)	(i) ITT 94.4 (90.6–98.2), PP 95.4, diff in proportion with LAB monotherapy 3.2 (8.15–1.80); (ii) ITT 99.4 (98.2–100), PP 100, diff in proportion with LAB monotherapy 1.3 (0.88–3.44); (iii) ITT 97.9 (95.5–100), PP 97.8, diff in proportion with LAB monotherapy 0.8 (4.60–3.04); (iv) ITT 98.1 (96.0–100), PP 98.7	39; NCT01122771
Positive rK39 test and parasitologically confirmed via bone marrow or spleen aspirate specimens	Initial cure = absence of fever, clinical improvement, reduction in spleen size, and no parasites in spleen or bone-marrow smears on day 45; final cure at 6 mo = clinical improvement with no signs or symptoms of relapse	(i) ITT 93.0 (87.5–96.3), PP 98.6 (94.7–99.8); (ii) ITT 97.5 (93.3–99.2), PP 98.7 (95.0–99.8); (iii) ITT 97.5 (93.2–99.2), PP 98.7 (94.9–99.8); (iv) ITT 98.7 (95.1–99.8), PP 98.7 (95.0–99.8)	38; NCT00696969
Positive rK39 test and parasitologically confirmed via bone marrow or spleen aspirate specimens	Initial cure = clinical improvement with no parasites at EoT or parasite density of 1 at EoT with no parasites on repeated smear 1 mo after EoT and no relapse during follow-up	(i) ITT 94.6; (ii) ITT 98.8, diff in cure rates 4.2, upper bound of 97.5% CI 6.9	25; NCT00216346
Fever for more than 2 wks, splenomegaly, rK39 rapid test positivity, and hemoglobin concn ≥50 g/liter	Initial cure = resolution of fever, decrease in spleen size, and increase in hemoglobin by 10% compared with baseline or to ≥100 g/liter; final cure at 6 mo = clinical improvement with no symptoms of relapse; spleen aspirate specimen confirmation in case of VL relapse	ITT 97, PP 98	35; CTRN1261200036784
VL symptoms and parasitologically confirmed via spleen aspirate specimens	Initial cure at EoT = clinical improvement and spleen aspirate specimen confirmation; final cure at 6 mo = clinical improvement with no signs or symptoms of relapse (spleen aspirate specimen confirmation in case of VL relapse)	(i) ITT 94 (91–97); (ii) ITT 97 (91–99)	18
VL symptoms and parasitologically confirmed via spleen aspirate specimens	Initial cure at EoT = resolution of fever, decrease in spleen size, return of laboratory values to normal ranges, and absence of parasites in spleen aspirate smears at day 29 (or at day 44 for those with a grade of ≥1); final cure at 6 mo = absence of signs and symptoms of relapse (spleen aspirate confirmation in case of VL relapse)	ITT 90.3 (87.9–92.7)	20
Fever for more than 2 weeks, splenomegaly, rK39 rapid test positivity, and hemoglobin level ≥6 g/dl	Initial cure at EoT or up to 2 mo after EoT = loss of fever, spleen size at least 30% smaller than at pretreatment, and Hb >10.0 g/dl or >10% higher than at pretreatment; final cure at 6 mo = loss of fever, spleen size at least 70% smaller than at pretreatment or, if spleen size was <1 cm at pretreatment, not >1 cm, wt gain ≥0.5 kg, and Hb >10.0 g/dl or increased ≥10% with respect to pretreatment value	ITT 71.8 (69.0–74.6),[g] PP 85	40
Confirmed VL diagnosis	Initial cure at EoT = parasitologically confirmed via spleen aspirate specimens; final cure at 6 mo = clinical improvement with no signs or symptoms of relapse	ITT 81.9 (79.6–84.1),[g] PP 95	41
Fever for more than 2 wks, splenomegaly, and parasitologically confirmed via bone marrow or spleen aspirate specimens	Initial cure at EoT = clinical improvement and bone marrow aspirate confirmation; final cure at 6 and 12 mo = clinical improvement with no signs or symptoms of relapse	At 6 mo, ITT 82.5, relapse rate 10.8 (5.2–16.4); at 12 mo, ITT 73.3, relapse rate 20.0 (12.8–27.2)	21
VL symptoms and parasitologically confirmed via spleen aspirate specimens	Initial cure at EoT = clinical improvement and spleen aspirate specimen confirmation; final cure at 6 mo = clinical improvement with no signs or symptoms of relapse	(i) ITT 95.7 (93.4–97.9), PP 95.7 (93.4–97.9); (ii) ITT 96.3 (92.6–99.9), PP 98.1 (95.5–100.0)	33; NCT00628719

outside on the ground and are exposed to sand flies, which increases the risk of infection. The majority of patients are children in charge of cattle and sleeping outdoors (42), with the exception of north Ethiopia, where the disease affects mainly young male adults living in a work-related setting (43).

Over the last few decades, a 30-day SSG regimen has been the mainstay of chemotherapy for treatment of VL in Eastern Africa. This 30-day monotherapy regimen has limitations: a dose-dependent antimonial toxicity, painful injections, and a long

period of hospitalization that has a high economic burden for the families of patients and results in a high occupancy rate of beds in hospitals. This favors mismanagement of patients, who are discharged before completion of treatment, which may increase the risk of resistance developing. Based on the 94.6% cure rate achieved in India (25), PM was tested as a monotherapy in Eastern Africa (15 mg/kg/day for 21 days), but the overall efficacy was only 63.8%, with substantial differences across the study areas: sites in Sudan had significantly lower levels of efficacy (14.3 and 46.7%), those seen in Gondar (northern Ethiopia) and Kenya were higher and similar (75% and 80%, respectively), while at Arba Minch (southern Ethiopia), the efficacy was 96.6% (44). The pharmacokinetic profiles established in subsequent studies do not seem to explain the observed difference (unpublished data).

In this same study, PM was combined with SSG with the aim of reducing the treatment duration and associated side effects of SSG monotherapy and to improve efficacy. In 2007, Mèdecins sans Frontières (MSF) showed that the SSG-PM combination reduced treatment duration from 30 to 17 days, a great improvement, but this being a combination of two injectable drugs, the treatment is still far from ideal (45). In a later randomized trial, the efficacy of this combination was 91% at 6 months, which is noninferior to SSG monotherapy (difference of 2.5%), but with the safety limitations inherent to each drug as mentioned before (46). This regimen has been recommended by WHO since 2010 and adopted by national control programs in all Eastern African countries where VL is endemic. A pharmacovigilance program in which 3,126 VL patients were treated with SSG-PM in 12 facilities in Ethiopia, Kenya, Sudan, and Uganda has confirmed no new safety signals, a high effectiveness at the EoT (95.1%), and a low mortality of 0.9%. However, results from this program also showed that SSG-PM is not a suitable treatment for patients who are >50 years old and HIV-VL-coinfected patients due to its lower efficacy and higher mortality rate, although these represent a small proportion of all VL patients (47). In Sudan, MSF found that the efficacy of SSG-PM treatment was 86% (506/591) at 6 months. Relapse cases had a higher risk of initial failure, and children <5 years old had higher risk of relapse during the follow-up period (48). The implementation of SSG-PM at the field level has been boosted recently by the implementation of a plan to strengthen the national control programs through the Ministries of Health, WHO, KalaCORE consortium (www.kalacore.org), and other local and international stakeholders.

Although SSG-PM combination therapy is an improvement over SSG monotherapy, a new therapy for VL should ideally be a safer, more efficacious, and shorter-course oral combination regimen more suitable for implementation in the remote locations of the populations affected in Eastern Africa. Affordability is also key to ensuring access to treatment.

Other drugs have also been tested in Eastern Africa. LAB was assessed in a clinical trial conducted in Gedaref State (Sudan) and in Gondar and Arba Minch (north and south Ethiopia, respectively). The trial aimed to identify the minimum efficacious and safe dosing regimen of LAB for future use in a short treatment regimen in Eastern Africa (49). The trial was terminated due to low efficacy observed in all study arms. Definitive cure rates were 85% in the LAB multiple-dose arm (total dose of 21 mg/kg), 40% in the single-dose 7.5-mg/kg arm, and 58% in the single-dose 10-mg/kg arm. Once more, an interregional difference was observed, with southern Ethiopia again presenting the highest cure rate (100% in patients treated with multiple doses or a single dose of 10 mg/kg), whereas Sudan and northern Ethiopia had similarly low efficacy rates (76% and 71% for multiple doses and 39% and 33% for the single 10-mg/kg dose, respectively) (49). Table 2 compiles the clinical trials carried out in Eastern Africa, including the diagnostic methods used to consider cure or failure.

Attempts to improve the efficacy of LAB by combining treatments failed in this region too. In a phase II clinical trial conducted in Kenya and Sudan, patients were treated with a combination of LAB (10 mg/kg in a single injection) and SSG (20 mg pentavalent sodium stibogluconate [Sb^{5+}]/kg/day for 10 days) or of LAB (10 mg/kg in a single injection) and MF (2.5 mg/kg/day for 10 days) or with MF monotherapy (2.5

TABLE 2 Recent visceral leishmaniasis clinical trials in Africa

Country(ies)	Site(s)	FPFV–LPLV (mo yr)[a]	Study design	Treatment(s) studied (drug[s], dosage[s])[b,c]	Total no. of patients enrolled or no. per arm[b]
Kenya, Ethiopia, Sudan	Kimalel, Gondar, Arba Minch, Um El Kher, Kassab	Nov 2004–Apr 2008	Randomized, open-label, parallel-group phase III trial	(i) PM 15 mg/kg/day for 21 days; (ii) SSG 20 mg/kg/day for 30 days; (iii) SSG 20 mg/kg/day + PM 15 mg/kg/day for 17 days	(i) 135, (ii) 135, (iii) 135
Sudan	Kassab	Oct 2005–Oct 2006	Randomized, open-label, dose-finding phase II trial	(i) PM 15 mg/kg/day for 28 days; (ii) PM 20 mg/kg/day for 21 days	(i) 21, (ii) 21
Kenya, Uganda, Ethiopia, Sudan	Kimalel, Amudat, Gondar, Arba Minch, Kassab, Um El Kher	Nov 2004–Jan 2010	Randomized, open-label, parallel-arm phase III trial	(i) PM 20 mg/kg/day for 21 days; (ii) SSG 20 mg/kg/day for 30 days; (iii) SSG 20 mg/kg/day + PM 15 mg/kg/day for 17 days	(i) 205, (ii) 386, (iii) 381
Ethiopia, Sudan	Arba Minch, Gondar, Kassab	May 2009–May 2011	Randomized, open-label, noninferiority phase II trial	(i) LAB 3 mg/kg on days 1–5, 14, and 21; (ii) LAB 7.5 mg/kg in single dose; (iii) LAB 10 mg/kg in single dose	(i) 63, (ii) 21, (iii) 40
Kenya, Sudan	Kimalel, Doka	May 2010–Oct 2012	Randomized, parallel-arm, open-label phase II trial	(i) LAB 10 m/kg in single dose + SSG 20 mg/kg/day for 10 days; (ii) LAB 10 m/kg in single dose + MIL 2.5 mg/kg/day for 10 days; (iii) MIL 2.5 mg/kg/day for 28 days	(i) 51, (ii) 49, (iii) 51
Kenya, Uganda, Ethiopia, Sudan	Kimalel, Kacheliba, Amudat, Arba Minch, Gondar, Abdurafi, Bazura, Dooka, Elhawata, Kassab, Um El Kher, Tabarakallah	Feb 2011–May 2014	Prospective phase IV pharmacovigilance cohort study	SSG 20 mg/kg/day + PM 15 mg/kg/day for 17 days	3,126
Sudan	Doka	Nov 2013–May 2014	Open-label, single-arm phase II proof-of-concept trial	FEX 1,800 mg q.d. for 4 days and then 1,200 mg q.d. for 6 days	14
Kenya, Uganda	Kacheliba, Amudat	Jun 2015–Mar 2016	Noncomparative, open-label, phase II proof-of-concept trial	MIL allometric dose for 28 days	30

[a]FPFV, first patient first visit; LPLV, last patient last visit.
[b]Data in different columns for different arms or time points are indicated by small Roman numerals.
[c]PM, paromomycin; SSG, sodium stibogluconate; LAB, liposomal amphotericin B (AmBisome); MIL, miltefosine; FEX, fexinidazole; q.d., once a day.
[d]EoT, end of treatment.
[e]95% CI, 95% confidence interval (not given in all cases); diff, difference; CC, complete case; ITT, intention to treat; PP, per protocol.
[f]End of treatment was the primary endpoint for this trial, and 6-month follow-up was not done.

mg/kg/day for 28 days). None of these treatments reached the target of 90% definitive cure rate at the 6-month follow-up, and further development was not deemed to be justified (efficacy rates were 87% for LAB-SSG, 77% for LAB-MF, and 72% for MF alone). The study did, however, show that according to the MF pharmacokinetic data, children were underexposed compared to adults, consistent with the lower efficacy in patients <12 years old (in children and adults, efficacy rates were 74% and 90%, respectively, with LAB-MF, versus 59% and 86% with MF monotherapy) (51).

As both studies were randomized clinical trials, a biased allocation does not explain the differences in response in the different study sites. There are two well-described ecological VL foci in Eastern Africa, one comprising eastern Sudan and northern Ethiopia, characterized by *Phlebotomus orientalis* as the main vector (52), associated with acacia-seyal and *Balanites aeyptiaca* trees that grow in cotton clay soils (53), and the other comprising the savannah and forest areas in southern Ethiopia, south Somalia, Kenya, and Uganda, where termite hills are important breeding sites for the main vectors, *Phlebotomus martini* and *Phlebotomus celiae* (53). In addition, PKDL manifests in an estimated 40 to 50% of patients in Sudan (54), while it is very uncommon in areas of Kenya and southern Ethiopia. Several factors could play a role in the differences observed in the Eastern African region—the different vectors, host factors (yet to be determined), and parasite diversity across the region. A remarkable genetic heterogeneity has been described among Eastern African strains of *L. donovani*, through genotyping of 14 highly polymorphic microsatellite markers (55). Eastern African *L. donovani* parasites are not only genetically distinct from the Indian strains,

TABLE 2 (continued)

Diagnostic criteria	Test of cure[b,d]	% efficacy (95% CI) at 6 mo follow-up per arm[b,e]	Reference; clinical trial no.
Parasite visualization in spleen, lymph node, or bone marrow aspirate specimens	Parasite clearance from spleen, bone marrow, or lymph node aspirate specimens at EoT and at 6 mo posttreatment	(i) CC 63.8, diff between SSG and PM = 28.5 (18.8–38.8); (ii) CC 92.2	44; NCT00255567
Parasite visualization in bone marrow aspirate specimens	Parasite clearance at EoT and at 6 mo posttreatment	(i) CC 81.0 (58.1–94.6); (ii) CC 80.0 (56.3–94.3)	50; NCT00255567
Parasite visualization in spleen, lymph node, or bone marrow aspirate specimens	Parasite clearance from spleen, bone marrow, or lymph node aspirate specimens at EoT and at 6 mo posttreatment	(i) ITT and PP 84.3, ITT diff between PM and SSG 9.7 (3.6–15.7), PP diff between PM and SSG 10.2 (4.2–16.2); (ii) ITT 93.9, diff between combination and SSG 2.5 (−1.3–6.3), PP 94.1, diff between combination and SSG 2.8 (−1.1–6.6); (iii) ITT and PP 91.4	46; NCT00255567
Parasite visualization in spleen, lymph node, or bone marrow aspirate specimens	Absence of parasites in spleen, bone marrow, or lymph node aspirate specimens at EoT and at 6 mo posttreatment (definitive cure)	(i) ITT and PP 85 (73–93); (ii) ITT and PP 40 (19–64); (iii) ITT and PP 58 (41–73)	49; NCT00832208
Parasite visualization in spleen, lymph node, or bone marrow aspirate specimens	Absence of parasites on microscopy of spleen, bone marrow, or lymph node aspirate specimens at day 28 (EoT); lack of VL signs or symptoms 6 mo after EoT and no requirement for rescue treatment during the trial	(i) ITT 87 (77–97); (ii) ITT 77 (64–90); (iii) ITT 85 (73–92)	51; NCT01067443
Clinical case definition + rK39 and, at 6 of the sites, parasitologic diagnosis was also performed	Treatment success = clinical cure and/or parasitological clearance on day 18 after treatment start (EoT) (clinical cure = fever clearance, improvement of disease signs and symptoms, improvement of hematological parameters, reduction of spleen size; microscopic examination of lymph node, spleen, or bone marrow aspirate specimens at day 18 was used for parasitology)	EoT 95.1 (94.4–95.9)[f]	47
Parasite visualization in lymph node or bone marrow aspirate specimens	Absence of parasites and no rescue treatment administered up to and including day 28; patients with no signs or symptoms of VL at day 210 who have not received rescue at any point in treatment or follow-up period	ITT 21 (5–51)	Unpublished data; NCT01980199
Parasite visualization in spleen, lymph node, or bone marrow aspirate specimens	Recovery of clinical signs and symptoms and absence of parasites at EoT (day 28); (ii) absence of signs and symptoms of VL at day 210 with no requirement for rescue medication during the trial	ITT and PP 90.0 (74–98)	Unpublished data; NCT02431143

but the parasites from Kenya and south Ethiopia are also distinctly different from those in Sudan and northwest Ethiopia. Further studies are needed to better understand the factors associated with these interregional differences, which is of paramount importance in the development of new tools to control VL in Eastern Africa.

As described above, exposure to MF was shown to be lower in children when MF was used either in monotherapy or in combination with LAB (51). Another trial was undertaken and demonstrated that efficacy in children could be improved by an allometric dosing regimen, comparable to the exposure in adults treated with conventional linear dosing of MF at 2.5 mg/kg/day. Thirty children suffering from kala-azar in Kenya and Uganda, aged 4 to 12 years, received MF in an allometric dosing regimen over 28 days—where weight, height, and sex were taken into account when calculating daily dosage—in an attempt to describe the pharmacokinetic profiles and compare them with historical data from patients treated with conventional linear dosing of MF at 2.5 mg/kg/day for 28 days. The treatment was very well tolerated, and the cure rate at the 210-day follow-up was 90%, similar to that previously described in adults. In addition, systemic exposure was slightly increased and less variable than with linear dosing (J. Mbui, J. Olobo, R. Omollo, A. Solomos, A. E. Kip, G. Kirigi, P. Sagaki, R. Kimutai, L. Were, T. Omollo, T. W. Egondi, M. Wasunna, J. Alvar, T. P. C. Dorlo, and F. Alves, submitted for publication).

In conclusion, all these observations point to the fact that it is more difficult to treat a VL patient in Eastern Africa than in South Asia, where similar treatments have an efficacy of >95%, except for SSG, for which resistant strains have been clearly described in India (Table 3). There are both inter- and intraregional variations in treatment efficacy in Eastern Africa.

Zoonotic Visceral Leishmaniasis

Zoonotic VL is caused by *L. infantum*, with canids as the main reservoirs, although

TABLE 3 Treatment efficacies in South Asia and Eastern Africa

Region	% efficacy(ies) (mean and/or range) (reference[s])[a]									
	SSG	LAB (20–21 mg/kg)	LAB (10 mg/kg in single dose)	MIL (2.5 mg/kg/day for 28 days)	PM (15 mg/kg/day for 21 days)	SSG + PM	LAB + SSG	LAB + MIL	PM + MIL	LAB + MIL
South Asia	35–95 (57)	>95 (36, 58)	≥95 (33, 35)	72–94 (18, 40)	94.6 (25)	NIA	NIA	>97 (38)	>97 (38)	>97 (38)
Eastern Africa	93.9 (46)	85 (71–100) (49)	58 (33–100) (49)	72 (51)	63.8 (14–96) (44)	91 (44, 46)	87 (51)	77 (51)	NIA	NIA

[a]Ranges are presented when there was a substantial interregional difference in efficacy. SSG, sodium stibogluconate; LAB, liposomal amphotericin B (AmBisome); MIL, miltefosine; PM, paromomycin; NIA, no information available.

other mammals, such as cats, rabbits, hares, bats, etc., are increasingly being implicated (59). The role of the dog in public health needs to be carefully considered when treating with the same drugs as for human leishmaniasis (60). Zoonotic VL is found both in the New World (South and Central America) and in the Old World (Africa, Europe, and Asia), specifically in four subepidemiological areas: the Mediterranean basin, Balkans, Caucasus, and Central Asia.

Human outbreaks of zoonotic VL are usually preceded by or concomitant with high infection rates in the canine population, as with the recently established transmission focus in northern Argentina (61) or the most affected area in northern Italy (62). Urban outbreaks were first described in Belo Horizonte, Brazil (63), but have also occurred in Europe, such as in Tbilisi, Georgia (64), and Madrid, Spain, where, surprisingly enough, the main reservoir was found to be the hare (65); urban outbreaks always cause social and political alarm.

Zoonotic VL has always been considered to be a lower priority in drug development than anthroponotic VL due to the smaller number of affected individuals. With the sharp decrease in the number of cases in the Indian continent, Brazil is now one of the top five countries with the highest number of annual cases, and there is an ever-increasing proportion of VL patients coinfected with HIV.

New World. In Latin America, *L. infantum* is the causal agent of zoonotic VL, and the domestic dog is the main reservoir of infection, with *Lutzomyia longipalpis* the most important vector. In Latin America, 52,176 cases were reported between 2001 and 2015, with an average of 3,835 cases registered per year, 96% of them in Brazil (66). Since the early 1980s, VL has expanded from rural areas in the northeast of Brazil to major urban centers in the north and, subsequently, to the south and west of Brazil. Currently, autochthonous cases are reported in 22 of 26 states. Mainstays of disease control are the identification of infected dogs and the use of insecticide-impregnated collars, dog vaccines, and culling, all approaches with controversial results. VL mortality increased during the period of 2000 to 2011 (67), and the current estimate is one of the highest in the world (7%) (9). Measures to improve disease control do not seem to have been effective in recent years.

The first-line therapy is MA over 20 to 30 days, based on WHO guidelines. A large multicenter randomized clinical trial was sponsored by the Ministry of Health (MoH) to assess the safety and efficacy of the recommended treatments of amphotericin B deoxycholate (1 mg/kg/day i.v. for 14 days), LAB (3 mg/kg/day i.v. for 7 days, total dose of 21 mg/kg), and a combination of LAB (10 mg/kg in an i.v. single dose) and MA (20 mg Sb^{5+}/kg/day i.v. for 10 days) compared to the first-line treatment of MA (20 mg Sb^{5+}/kg/day i.v. for 20 days). The ultimate goal was to provide the MoH with objective evidence to guide policy change. Based on safety data obtained in an interim safety analysis of this trial, a policy change was implemented, with LAB replacing amphotericin B deoxycholate as the second-line treatment, due to the high toxicity of the latter. Efficacy rates at 6 months were 77.5% for MA, 87.2% for LAB, and 83.9% for LAB-MA; no statistical differences were determined. However, LAB was safer than the two arms involving MA. Based on these results, the MoH is evaluating a change in policy such that LAB would be the first-line treatment in Brazil and MA the second-line treatment (68). Nevertheless, a cure rate of 87.2% is still not good enough, and more efficacious treatments are needed for the region. Table 4 summarizes the clinical studies carried out in Brazil, including the test of cure.

Old World. The WHO European region comprises 53 countries and covers a vast geographical region, extending from western Greenland to the Pacific shores of the Russian Federation and from the Baltic Sea to the Mediterranean: zoonotic VL is found in this region, as well as in China. The Mediterranean basin accounts for approximately 1,500 zoonotic VL cases per year, mainly children, with 5,000 cases occurring annually in the Balkans, Caucasus, and Central Asia: 80% of all cases are reported in Albania, Georgia, Greece, Italy, Spain, and Tajikistan. Zoonotic VL is found across an area extending from Portugal and Morocco to China, the latter having some 200 cases, 97% of them found in Xinjiang, Schuan, and Gansu provinces (71). The spread of zoonotic

TABLE 4 Visceral leishmaniasis clinical trials in Latin America

Country(ies)	Sites	FPFV–LPLV (mo yr)[a,d]	Study design	Treatment(s) studied (drug[s], dose[s])[b,c]	No. of patients per arm[b]	Diagnostic criteria	Test of cure	% efficacy (95% CI) for indicated analysis per arm[b,d]	Reference; clinical trial no.
Brazil	Federal University of Piaui', Teresina; Montes Claros State University, Montes Claros; Pediatric Hospital Joao Paulo II–FHEMIG, Belo Horizonte; Federal University of Sergipe, Aracaju; Sao José Hospital of Infectious Diseases, Fortaleza, Ceara	Jan 2011–Oct 2014	Open-label, randomized, controlled trial	(i) MA 20 mg Sb^{5+}/kg/day for 20 days; (ii) Amp B 1 mg/kg/day for 14 days; (iii) LAB 3 mg/kg/day for 7 days; (iv) LAB 10 mg/kg/day in single dose on first day + MA 20 mg Sb^{5+}/kg/day for 10 days	(i) 112, (ii) 45, (iii) 109, (iv) 112	Positive rK39 test and/or positive microscopic examination of bone marrow aspirate specimen or positive culture	Clinical cure at 6 mo follow-up = complete remission of clinical signs and symptoms up to 3 mo after beginning of treatment, associated with normalization of hematological abnormalities observed at baseline, without evidence of relapse up to 6 mo	(i) ITT 77.5, PP 94.5; (ii) NI; (iii) ITT 87.2, diff in cure rate with MA 9.7 (0.28–19.68), PP 92.2, diff in cure rate with MA 2.3 (9.23–4.60); (iv) ITT 83.9, diff in cure rate with MA 6.4 (3.93–16.73), PP 98.9, diff in cure rate with MA 4.4 (0.73–9.53)	68; NCT01310738
Brazil	Dona Regina Hospital, Palmas; Tropical Diseases Hospital, Araguaína, Tocantins	Jan 2006–Jan 2009	Open-label, randomized, controlled trial	(i) MA 20 mg/kg/day for 20 days; (ii) Amp B 1 mg/kg/day for 14 days	(i) 51, (ii) 50	Direct bone marrow microscopic examination or parasitic DNA detection by PCR	Clinical cure at day 180 = complete remission of clinical signs and symptoms up to 3 mo after treatment completion, normalization of hematological changes, and no recurrence of VL up to 6 mo follow-up	(i) ITT 94.1 (84.1–97.9), PP 100 (92.5–100); (ii) ITT 94.0 (83.8–97.9), PP 97.9 (89.1–99.6)	69; NCT01032187
Brazil, Kenya, India	Hospital Universitario Professor Edgard Santos, Salvador, Bahia, Brazil; Kenya Medical Research Institute, Nairobi, Kenya; Patna Medical College and Hospital, Patna, Bihar; India	NI	Open-label phase II, randomized, controlled trial	(i) LAB 2 mg/kg/day on days 1–6 and 10, total dose 14 mg/kg; (ii) LAB 2 mg/kg/day on days 1–4 and 10, total dose 10 mg/kg; (iii) LAB 2 mg/kg/day on days 1, 5, and 10, total dose, 6 mg/kg; (iv) LAB 2 mg/kg/day on days 1–10, total dose, 20 mg/kg	(i) Brazil, 13; Kenya, 10; India, 10; (ii) Brazil, 4; Kenya,10; India, 10; (iii) Brazil, 0; Kenya, 5; India, 10; (iv) Brazil, 15; Kenya, 0; India, 9	Direct examination of spleen (rarely bone marrow) aspirate specimens	Initial cure = parasitological diagnosis at 0.5 mo follow-up; clinical cure = improvement in physical examination and laboratory parameters at 0.5, 2, and 6 mo	(i) Brazil, 62 (no 95% CI available); Kenya, 100; India, 100; (ii) Brazil, 100; Kenya, 90.0; India, 100; (iii) Brazil, NA; Kenya, 20.0; India, 100; (iv) Brazil, 87.0; Kenya, NA; India, NA	70

[a]FPFV, first patient first visit; LPLV, last patient last visit.

[b]Data in different columns for different arms are indicated by small Roman numerals.

[c]MA, meglumine antimoniate; Sb^{5+}, pentavalent antimony; Amp B, amphotericin B deoxycholate; LAB, liposomal amphotericin B (AmBisome).

[d]NI, no information; 95% CI, 95% confidence interval (not given in all cases); ITT, intention to treat; PP, per protocol; diff, difference; NA, not applicable.

VL is associated with human and dog migration from areas of nonendemicity to areas of endemicity, climate change (sand flies and vectors invading previously noncolonized areas), and increasing numbers of cases of acquired immunosuppression (72). Leishmaniasis and immunosuppression is of increasing importance for two reasons. First, *Leishmania*-and-HIV coinfection is spreading due to overlapping of the former, progressing from rural transmission toward urbanization, with the latter as it invades rural areas (3, 73). Second, surgery involving transplantation of solid organs is becoming more frequent, along with other diseases that require immunosuppressive or immunomodulatory treatments, such as anti-TNF-α therapy (74).

In Old World zoonotic VL, evidence to support treatment recommendations is sparse, and therefore, recommendations are inconsistent (75). Despite the lack of clinical trial data, MA has been the drug of choice for decades, having a cure rate of 95%; it is better tolerated in children than in adults (76). A retrospective analysis of 1,210 children in Albania (1995 to 2009) demonstrated that MA given at 20 mg/kg for 21 to 28 days was 99% effective (77). The same lack of information applies to MF, PM, and amphotericin B deoxycholate. Although a randomized clinical trial has yet to be done, an open-label dose-finding study with LAB demonstrated 100% efficacy with a total dose of 24 mg/kg, 97.6% with a total dose of 18 mg/kg, and 90.6% with a total dose of 15 mg/kg, administered over 5 consecutive days plus one more infusion at day 10 in all three cases (78). A study in Greece using a total dose of 20 mg/kg LAB demonstrated an efficacy of 97.6% when administered over 2 days versus 90.4% when given over 5 days (79). The current recommendation in the Mediterranean region for LAB is 3 to 5 mg/kg daily, given over 3 to 6 days (total dose of 18 to 21 mg/kg) (80).

PERSPECTIVES

As reviewed herein, the current medicines, including the combinations, are far from the optimal target product profile, leaving uncovered a number of needs in the treatment of VL (Table 5).

Ideally, the priority in leishmaniasis is to develop an efficacious, safe, oral, and affordable short-course combination treatment for patients with VL, preferably as a drug combination to combat any potential for resistance (Table 5). In this regard, a tablet with a fixed-dose combination would be preferable to the coadministration of two oral drugs, and if possible, not needing direct observation of treatment. There are a number of compounds in the very early stages of screening, hits to lead, lead optimization, and in lesser degree, at the preclinical stages being explored by different companies or groups of researchers. Those completing the final preclinical stages that eventually can move to phase I studies are cited here and belong to different pharmaceutical companies or universities. On the other hand, the open-access Pathogen Box collection, which contains approximately 400 diverse, druglike molecules with demonstrated biological activity against specific pathogens, provides new chemical starting points for a number of tropical and neglected diseases, through repurposing of these compounds for use in drug discovery campaigns for different pathogens (https://www.pathogenbox.org/screening-medicines-malaria-venture-pathogen-box-across-multiple-pathogens-reclassifies-starting). For the first time ever, there is now a rich portfolio of orally administered drugs identified by DND*i* and/or its partners, comprising six drug candidates from five different classes, all with excellent anti-*Leishmania* activity. Four of these candidates are currently at the preclinical development stage, whereas two have been nominated to clinical candidate status and will have phase I studies initiated in healthy volunteers in late 2018. These candidates share important properties to become new treatment(s) for VL and other leishmanial forms. They all share the potential to act as orally dosed drugs and achieve >95% reduction of parasite load in animal models of visceral leishmaniasis after 10 days or less of treatment as a monotherapy. Among these six candidates, the mechanism of action has been identified for four, two of which share the same mechanism of action. It is hoped that combinations of such drugs with different mechanisms of action may achieve faster and greater reductions in parasitemia in

TABLE 5 Unmet needs in the treatment of VL and principles to develop NCEs[a]

Unmet need(s)	Guiding principles for the development of NCEs
The VL incidence has changed recently in Asia, with a sharp decrease in the number of cases; in Africa, treatment requires two painful and toxic injections per day for 17 days	Prioritize by geographical region: start in Eastern Africa and expand to Latin America and Asia
In Africa and Latin America, the efficacy of the best therapeutic option is ~ 90%	A treatment that is highly efficacious in all regions is needed—clinical efficacy should be ≥95% regardless of the *Leishmania* species, although regimen adaptations may be required in the different regions; the primary efficacy endpoint remains cure at 6 mo
In terms of safety and tolerability, the cardiac, hepatic, renal, or gastrointestinal toxicity or teratogenic risk of the existing medicines require close monitoring of the patient	The need is for an efficacious, safe, and field-adapted treatment not requiring adverse event monitoring to be deployed in the remote areas where VL affects rural communities
In Africa and Asia, not all treatments prevent the development of PKDL	Ideally, a treatment covering VL and PKDL, or even better, a VL treatment with a high cure rate and not evolving to PKDL
VL is often associated with HIV infection, leading to frequent relapses and threatening patients' lives	A treatment that would be efficacious in both immunocompetent and immunosuppressed patients
In VL transmission areas, HIV, tuberculosis, and malaria frequently coexist, complicating patient management	No interactions between the drugs to be combined for VL treatment or for treatments for other common comorbidities (malaria and tuberculosis drugs, antiretroviral therapy)
An ideal treatment should combine two oral drugs, but two oral drugs are not yet available; also, according to its PK, MIL, the only existing oral drug, requires a minimum of 2 weeks administration, which implies possible compliance problems.	Monotherapy vs combination: assess each drug as monotherapy, but aim for combination in the development process, as well as for the possibility of short treatment duration (ideally in one single dose or a fixed-combination tablet), an acceptable safety profile, and ideally, different mechanisms of action; PK/PD relationship to guide the process of development
Although over 60% of all patients worldwide are children, there are not pediatric formulations	Pediatric study plans: children should be the main target population for the new VL treatment, which should have a duration of up to 7 days, although up to 14 days is acceptable; the phase III trial should include children ≥6 yr old
Stability is relatively good in most of the existing medicines, excluding LAB, which requires a cold chain	Stable for ≥2 yr in zone 4 without a cold chain

[a]NCEs, new chemical entities; PKDL, post-kala-azar dermal leishmaniasis; PK, pharmacokinetics; PD, pharmacodynamics; MIL, miltefosine; LAB, liposomal amphotericin B (AmBisome).

human patients than the standard of care, offering a reduction in treatment duration below 10 days. Identification of drug partners for use in combination will involve *in vitro* and *in vivo* animal tests to assess the potential for efficacy in patients, as well as assessment of the potential for resistance development and cross-resistance to current therapy. In order to meet the objective of a new oral combination treatment and taking into account attrition during drug development, we calculate a minimum of 10 candidates will be required to bring at least one orally administered drug to registration phase. To meet this target, four more candidates coming from DND*i* internal projects, as well as the work of partners, are anticipated to reach preclinical candidate status during 2018 and 2019.

The five new classes of preclinical/clinical candidates are described in Fig. 1, accompanied by a short perspective on additional new classes that may provide further options for clinical evaluation in the coming decade.

Nitroimidazole Class

For more than 7 years, DND*i* has attempted to develop a new drug for leishmaniasis from the nitroimidazole class of drugs, which is known to have a broad spectrum of antiparasitic properties. Although the development of first fexinidazole (81) and then VL-2098 (82, 83) for treatment of VL was halted due to unsatisfactory results, the DND*i* team used this experience to select a potentially superior compound, DNDI-0690 (84), as a preclinical-development candidate in September 2015. This compound is highly effective in both mouse and hamster models of VL, with treatment durations of 3 to 10 days depending on the dose used. It is believed that the nitro group of DNDI-0690 is bioactivated by the nitroreductase enzyme NTR2 (85) in *Leishmania* parasites, leading to reactive intermediates that kill the parasite. The preclinical development of DNDI-

0690 is completed, it was nominated as a clinical candidate in January 2018, and a phase I ascending-single-dose study in healthy volunteers is expected to be initiated in mid-2018.

Oxaborole Class

DND*i* selected an oxaborole, DNDI-6148, for preclinical development in January 2016 (86). This candidate achieves high levels of parasite burden reduction in mouse and hamster models of VL after 5 to 10 days of treatment, depending on the dose level selected. Although the mechanism of action of DNDI-6148 (86) has not yet been determined, it is expected to be distinct from that of the antileishmanial therapies currently used, as it retains activity against strains resistant to those drugs. The regulatory safety pharmacology and toxicology studies have been completed, and DNDI-6148 has been nominated as a clinical candidate and will enter a phase I single-ascending-dose study in mid-2018.

Proteasome Inhibitor Class

In September 2016, a team led by Novartis researchers published impressive compound profiles and the elucidation of the mode of action of a novel class of *Leishmania* proteasome inhibitors (87). These molecules display remarkable *in vitro* and *in vivo* activity in a variety of preclinical models of VL and other kinetoplastid diseases. In May 2017, an optimized compound from this series, LXE408, was presented as a preclinical candidate at WorldLeish-6 (88). Provided that preclinical development of this candidate is completed successfully, it will provide an opportunity for clinical trials to evaluate this novel anti-*Leishmania* mechanism of action.

A team from GlaxoSmithKline Plc. (GSK) and the University of Dundee Drug Discovery Unit presented a second proteasome inhibitor candidate, GSK3494245/DDD1305143, from a different chemical series in September 2017 (M. Marco, personal communication), providing an additional potential chance to test this mode of action in the clinic. This candidate is in the late stage of preclinical development, with entry into phase I expected in early 2019.

CRK-12 Kinase Inhibitor Class

A year earlier, in September 2016 (90), the same team from GSK and the University of Dundee Drug Discovery Unit presented a novel preclinical candidate, GSK3186899/DDD853651, which is able to reduce parasite load by $>$95% in an acute mouse model of *Leishmania* infection following a 10-day oral treatment. The antiparasitic mechanism of action of this compound was subsequently shown to be due to inhibition of a parasitic kinase, CRK-12, by elegant work presented at WorldLeish-6 (91). If preclinical development proceeds successfully, this molecule will enable clinical evaluation of this novel mode of action.

Aminopyrazole Class

The initial discovery of a novel class of aminopyrazoles with excellent *in vitro* and *in vivo* activity in preclinical models of VL was reported by DND*i* in 2015 (92). An optimized compound, DND*i*-5561, was nominated for preclinical development in October 2017 (93). The mechanism of action of DND*i*-5561 has not yet been elucidated, but as for DND*i*-6148, it is believed to be distinct from that of existing treatments.

In addition to these six preclinical/clinical candidates, with most likely five different mechanisms of action, there have already been reports of further new classes of compounds undergoing optimization, such as a novel heterocyclic series (94) from Celgene and DND*i*, a family of dipeptidylcarboxypeptidase inhibitors (95) from the Central Drug Research Institute in India, and several other ongoing, but still confidential, programs. It is therefore plausible that 10 or more preclinical candidates will have advanced into preclinical development by the end of 2019. This provides an unprecedented opportunity to develop highly effective, short-course combination treatments for VL that should minimize the risk of resistance emerging to the individual new drugs.

This rich opportunity will require significant support from both the research community and donors to avoid delays in delivering much-needed and greatly improved new treatments for patients in need.

DISCUSSION

The Kala-azar Elimination Program (17) in South Asia has had an incontrovertible effect to date, with an overall 79% reduction of incidence (100% in Nepal for more than 2 years, 94% in Bangladesh, and 72% in India) and a decrease in mortality of 94% (96). Overall, the WHO elimination target of $<$1 case per 10,000 people per year at district and subdistrict levels has been achieved in 87% of the districts where VL is endemic. Improved case management, through early case detection and treatment, have contributed not only to saving lives but also to reducing transmission. Other pillars in the program, such as vector control, social mobilization, surveillance, and operational research, have certainly contributed to the decrease in the number of cases; however, the lack of baseline indicators precludes a detailed analysis of how each has contributed (97, 98). Moreover, the target of $<$1 case per 10,000 population at risk is an artificial figure taken from the Leprosy Eradication Program, which implies uncertainty for the future in the sense that transmission may not be interrupted (reproduction rate [R_0] of $>$1). The sharp decrease in the number of cases coincides with the natural fluctuation of VL incidence in South Asia, making it difficult to assess the impact of the program. Furthermore, the role of PKDL patients and asymptomatic carriers in fueling transmission needs to be better understood and is further complicated by the distribution of parasites observed in patches of skin in the mouse model (99). Consequently, more evidence is needed to allow for solid output from mathematical modeling in order to predict the natural history of transmission and, therefore, the program's sustainability (100). Meanwhile, as the attack phase transitions to the consolidation phase in the Kala-azar Elimination Program, new foci or pockets of patients are being identified, delaying the elimination target (101).

Direct observation of parasites by microscopy is the gold standard method, and it has always been used as an inclusion criterion and for test of cure at the EoT or day 45, defined as initial cure. Final cure at 6 months was defined clinically with no signs and symptoms of VL. Parasitological confirmation is done by either bone marrow or spleen aspiration, which differ in sensitivity due to a higher parasite load in the latter. Sensitivity may be limited when very few parasites remain, especially at the EoT. Indeed, differences in the sensitivity of the methods used to evaluate cure could impact the results of a trial, and therefore, assessment of relapses after 6 months is important; in fact, MSF and DND*i* observed additional relapses occurring between 6 and 12 months, justifying the extension of follow-up to 12 months in future studies. In other words, test of cure assessed by microscopic examination may be insufficiently sensitive and, thus, miss some treatment failures. Levels of parasites undetectable by microscopy may be revealed with a more sensitive method, such as quantitative PCR (qPCR), but this still needs to be validated as a reliable new method to assess cure, which may also detect false positives if parasitic DNA is still present. In Tables 1, 2, and 4, the tests of cure used in different trials are specified. It is difficult to analyze the results across the trials, because initial cure was evaluated at the EoT, at day 45, or at 2 months depending on the trial. Moreover, although microscopy is the method used, the tissue aspirated influences the results. Even bearing in mind that it is based only on symptomatology, follow-up at 6 (and 12) months is critical to determining patient cure and, therefore, the cure rate of a given treatment: it remains the primary efficacy endpoint to account for risk of relapse after initial cure at the EoT. In order to answer the unresolved limitations of diagnosis, the Afri-KA-Dia consortium has been created. It is supported by the European Union-European & Developing Countries Clinical Trials Partnership (EU-EDCTP) and is a platform comprising four African countries and 6 European teams. The context is a recently started, ongoing phase III clinical trial (ClinicalTrials registration no. NCT03129646), which aims to develop a safe, efficacious, field-adapted new combination therapy for primary VL patients in Eastern Africa by 2020. Under this umbrella, the

second objective is to evaluate less invasive and more sensitive tools for diagnosis (rK39 versus rK28), prognosis (IgG1 and cytokine profile), and monitoring of EoT (loop-mediated isothermal amplification [LAMP] versus tissue aspirates and PCR) for support of VL treatments in clinical trials in Eastern Africa.

As for diagnosis, the sensitivity of RDTs varies according to geographical region, being less efficacious in Africa and Latin America than in Asia (10, 11). With the target of VL elimination, the inconsistent performance of RDTs in different regions, and the lack of a test of cure, better tools are urgently needed to control the disease. The identification of new biomarkers (parasitic or human) to demonstrate the presence/absence of parasites or to predict treatment failure and relapse has been repeatedly identified as a top priority (1, 12, 102, 103).

In South Asia, all VL treatment regimens, either monotherapies or combinations, have shown excellent cure rates in initial clinical trials (104). However, as with MF monotherapy, efficacy rates are decreasing with time and relapse rates are increasing, with a 2-fold increase in relapse rate observed in India after just 1 decade of use (20) and relapse rates reaching 20% after the 12-month follow-up in Nepal (21). The analysis of pharmacokinetic parameters showed that there was a decreased risk of failure with time when the MF concentration was above 10 times the 50% inhibitory concentration (IC_{50}; 17.9 μg/ml). Increased risk of failure was associated with age (children 2 to 9 years old and 10 to 14 years old had 3.2- and 2.5-fold-higher risks of failure than adults, respectively) and male patients (105). This is consistent with the ~30%-lower MF exposure in children than in adults at the EoT (using the standard dosage at 2.5 mg/kg/day for 28 days) (22). However, these data prompt dosage adjustment in children to achieve exposure to MF at levels comparable to those in adults. In practice, MF was sold over the counter in the Indian private market at the beginning of the KAEP at an inflated price (19), compromising compliance with the 28-day treatment. From patient and programmatic perspectives, it was important to remove SSG and MF monotherapies from use. Finally, in August 2014, the Indian Government adopted a single infusion of LAB as the first option for the KAEP. Since 2015, there has been a substantial investment by the United Kingdom's Department for International Development (DFID) to deploy LAB through the KalaCORE program, by improving the cold chain infrastructure in health centers and by training health personnel in case management (http://www.kalacore.org/). The second option chosen—the MF-PM combination—is cold chain independent and, therefore, can be deployed in remote areas where electricity is a problem or where the small number of cases does not justify the purchase of cooling equipment or training of personnel. However, the sustainability of the KAEP will be a challenge post-2018 if political commitment declines, in which case the MF-PM combination will become more relevant for the consolidation and maintenance phases.

In the short term, surveillance is crucial in order to sustain the achievements already made. Surveillance should include pharmacovigilance at sentinel sites to identify the safety profile of all regimens at the field level and drug resistance/susceptibility. The elements which fuel transmission are not yet fully understood, and it is essential to elucidate the role of PKDL patients and asymptomatic carriers as reservoirs of the parasite (106, 107). Once available, this evidence should guide further research and development of new treatments (if found to be necessary for asymptomatic carriers) that are compatible with the risks and benefits for these two specific populations. It will require a very safe, efficacious, and ideally, short-course oral treatment that can be easily implemented in the field. Treatment will not only benefit individuals but has the potential to be a preventative tool for use in public health, capable of reducing the transmission of anthroponotic VL in the case of reemergence.

In Eastern Africa, the scenario is different. The efficacies of all drug treatments are lower than in Asia, with the exception of SSG, due to the high rate of resistance in Asia. The SSG-PM combination is the first choice in the region (1), with an efficacy of 91% at 6 months, but it requires 17 days of hospitalization (46). Effectiveness data showed that this regimen is more toxic and results in greater mortality in patients

>50 years old and in coinfected HIV-VL patients (47). Trained health personnel and upgraded hospitals are key to minimizing the limitations of this regimen until a new (ideally oral) combination is ready. As a general rule, patients are obliged to travel long distances to seek treatment, and mortality increases when access to health care is delayed (108). The natural incidence peaks every 6 to 10 years, and it is important to anticipate and prepare for these surges in patient numbers for proper management, as funds tend to be diverted to competing needs in other health sectors between epidemics.

Ministries of Health also need to commit to improving access to treatment. In a recent survey, it was shown that only 7 of 35 centers providing kala-azar treatment in Sudan were using SSG-PM, which was recommended by WHO in 2010 (KalaCORE, unpublished data in 2015) and is the first-line treatment in Sudanese guidelines. Mobile teams trained in case management receive ongoing mentoring by specialized health workers, with the result that SSG-PM is used globally in Eastern Africa. In Ethiopia, KalaCORE's three mentoring teams have completed more than 50 rounds of visits to all hospitals providing diagnosis and treatment for VL, and an estimated 1,000 health workers have now received on-site mentoring. This intervention has had a very significant positive impact on the quality of diagnosis and treatment of VL in the region. In South Sudan, two mobile teams, in over 100 mentoring visits to often very remote facilities, have given on-site training and mentoring to over 400 health staff. These mobile teams also bring supplies (drugs and diagnostics), provide information education communication (IEC)/behavior change communication (BCC), and collect reporting figures during their visits. The activities of the teams have been essential for improving access to diagnosis and treatment of VL in this country, where in many cases, there was none, and under circumstances of war.

Semi-immunity in areas of endemicity develops with age, and subsequent herd immunity protects communities against outbreaks (109). However, VL-naive populations are at risk from epidemics, facilitating the geographical spread of disease. Epidemics occur every 10 years or so and are usually associated with poverty, population displacement, malnutrition, collapse of health infrastructures, unplanned urbanization, and environmental changes (110). Civil unrest in South Sudan has led to internal displacement of immunologically naive populations, who eventually arrive in areas of active transmission (111). Not only does outbreak preparedness require a concerted response among the various stakeholders in order to control the spread of cases and reduce mortality, but there is also an urgent need for shorter-course treatments, which ultimately do not require patient hospitalization. During the outbreak in Unity, Jonglei, and Eastern Upper Nile States in South Sudan in 2010 to 2012, a rapid response to the almost 30,000 VL cases kept the reported mortality rate down to 4%, significantly lower than the 35% mortality resulting from the previous VL outbreak in the 1990s (112). Keeping a small network of sentinel VL treatment centers integrated in the primary health structure allowed early detection and response to the increase in VL incidence (113). Injectable treatments are not the best option for this, and again, an oral short-course combination treatment would be ideal. There is an ongoing effort to improve VL surveillance by strengthening national control programs and collecting individual data records at the district level. This would improve outbreak preparedness and early identification of hot spots of the disease (114).

In Africa, the current drugs showed lower rates of efficacy than in Asia, which could be related to differences in parasite populations and drug susceptibilities, host factors like immunological response, or pharmacokinetic profiles, as illustrated by low concentrations of MF in children <12 years old at the EoT. After the various attempts made by DND*i* and the Leishmaniasis East African Platform (LEAP) to assess the safety, efficacy, pharmacokinetics, and pharmacodynamics of LAB-SSG, LAB-MF, and MF alone, the rates of efficacy were found to be insufficient to justify progression to a phase III trial compared with the standard SSG-PM regimen (51). The pharmacokinetics findings were crucial, however, demonstrating underexposure to MF in African children compared to the exposure in adults (the efficacy rates of MF monotherapy in children and adults

were 59% and 86%, respectively) (51). This prompted the implementation of a new clinical trial in the region, in which the pharmacokinetics, safety, and efficacy of an allometric regimen for MF were evaluated in pediatric patients (4 to 12 years) (Mbui et al., submitted), resulting in substantial increases in efficacy in this age group, from 60% (in Sudan) and 57% (in Kenya) treated with the conventional 2.5-mg/kg/day regimen to 90% (in Kenya and Uganda) when allometric dosing was used. It is worth noting that more than 65% of patients in Africa are children (42).

A sustainable cold chain in areas of transmission in Africa is only feasible in referral hospitals, and therefore, LAB use is limited. Taking into consideration all studies undertaken in Eastern Africa, the only treatment with currently available drugs that could improve on the current SSG-PM therapy is MF-PM, which is the highly efficacious and safe combination treatment adopted in Asia. Based on the efficacy and safety data for PM from the development of the SSG-PM combination and the MF allometric study in children, an adapted regimen of 20 mg/kg/day PM for 14 days in combination with MF over 14 or 28 days of treatment will be assessed. If it proves to be noninferior to SSG-PM, it will be an attractive option in Eastern Africa, removing the need for toxic and painful SSG injections and allowing shorter hospitalization periods, making it particularly suitable for children and elderly patients. This trial started in December 2017 in four Eastern African countries and is being carried out by members of the Leishmaniasis East African Platform (LEAP). Nevertheless, as for Asia, new oral treatments that are safe, efficacious, and adapted to the field are of utmost importance for the control of VL in Eastern Africa.

In Brazil, treatment options are very limited. MA remains for the moment the first-line treatment, despite its highly toxic profile, causing permanent treatment discontinuation in more than 10% of cases treated (115). Although it has not been established, antimonial toxicity could be one of the factors associated with the high mortality rate in Brazil, one of the highest in the world (67). LAB is the second-line treatment and has broad indications, which are estimated to cover at least 30% of cases reported. In addition, patients who cannot tolerate MA are to be switched to LAB, increasing its use to more than 40% of VL patients in the country. Given its much better safety profile, shorter hospitalization period (7 days), and the number of cases reported in Brazil (~3,500 cases/year), it would be justifiable to consider this as a much better option for VL patients. A cost-effectiveness analysis of the treatment options could provide additional information to inform decision making. Furthermore, the total dose currently recommended in Brazil for primary VL is 21 mg/kg for the complete treatment. This compares to the situation in Eastern Africa, where the total dose is 30 mg/kg over 10 days and up to 40 mg/kg for HIV-VL patients. It may be that the efficacy of LAB in Brazil could be improved by adjusting the total dose while still maintaining the short treatment duration (for example, a dose of 4 mg/kg/day for 7 days).

The potential usefulness of MF for VL treatment in Brazil is still unclear. A clinical trial to assess the efficacy and safety of MF (ClinicalTrials registration no. NCT00378495) was conducted in 2005 in Teresina and Montes Claros, although it was never published. It showed unsatisfactory efficacies of 43% at 6 months in patients treated with 2.5 mg/kg/day for 28 days and of 67% in patients treated with the same regimen for 42 days (116), which resulted in the early termination of the trial. This lower cure rate observed in Brazil compared to the results obtained in India may be attributed to a lower susceptibility of the parasite species involved and/or, possibly, to MF underexposure, both in adults and children. Of note, 40% of the VL patients in Brazil are children under 10 years of age (66). Interestingly, dogs naturally infected with *L. infantum* in the high-endemicity state of Piaui were treated with three different regimens of MF, and although some clinical improvement was observed, the treatments were not sufficient to clear parasites (117).

In conclusion, an innovative, field-adapted treatment is greatly needed in Africa and Latin America for disease control and in Asia to sustain the elimination program. The target product profile for an ideal treatment is an oral, safe, highly efficacious combination treatment for all regions that can be given over a short period of time (≤2

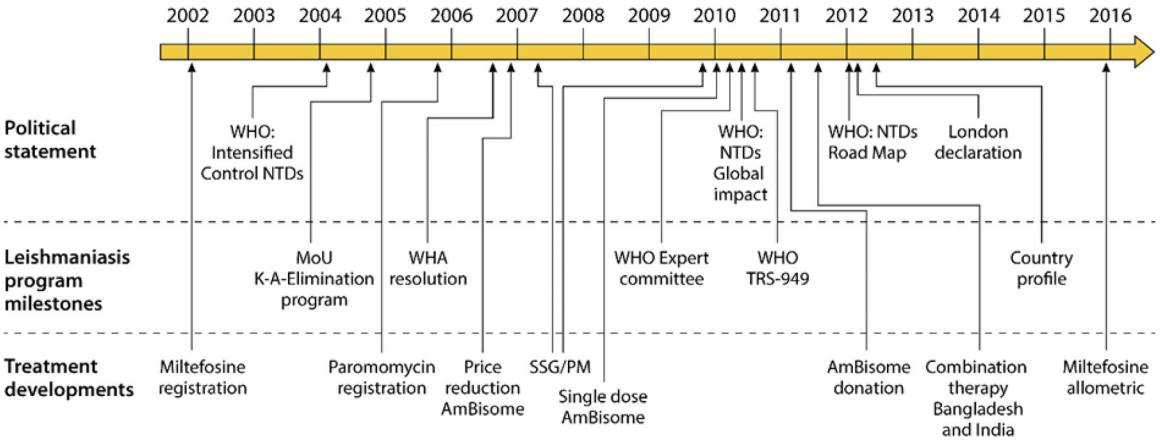

FIG 2 Chronogram of benchmarks in visceral leishmaniasis.

weeks) and is accessible in remote areas where VL occurs. In order to ensure access, treatment also needs to be affordable (118).

FINAL REMARKS

The past 15 years have seen great changes in VL from the political and scientific points of view. The licensing of MF in 2002 as the first oral drug to treat VL in India generated an enthusiasm among Asian politicians, who finally signed a memorandum of understanding to eliminate VL by 2015 in Bangladesh, India, and Nepal. For the first time ever, in 2007, the World Health Assembly approved a resolution to combat this disease, providing the umbrella to unite efforts between countries, WHO, academia, stakeholders, and philanthropic organizations, encouraging the development of new treatments to control VL (119). The price of LAB was reduced by 90% in 2007 for low- and middle-income countries of endemicity, opening a new therapeutic opportunity. In 2010, the WHO Expert Committee met, after a 20-year gap, to update the worldwide therapeutic guidelines that were subsequently adopted region by region (1). All medicines for VL were progressively incorporated into the Essential Medicines List by 2012, a key step for facilitating registration by regulatory bodies at the national level. Finally, the publication in 2012 of new epidemiological figures for VL provided detailed information on the disease burden at the country level. This new scenario raised the interest of stakeholders and donors in combating leishmaniasis from almost nothing to the point where VL was incorporated into the London Declaration, to be eliminated by 2020 from the Indian subcontinent (http://www.who.int/neglected_diseases/London_Declaration_NTDs.pdf).

As shown in Fig. 2, there were in parallel advances in the science, with clear milestones in the process of bringing new medicines to the existing limited selection, namely, oral MF in 2002, i.m. PM in 2005, a single-dose liposomal formulation of amphotericin B in 2010, and a series of clinical trials assessing combinations of the existing medicines by regions and by clinical forms (VL, HIV-VL, and PKDL). Many lessons were learned in this endeavor: inter- and intraregional differences prevented the extrapolation of results from clinical studies between Asia and Africa, making the process of clinical research more complicated, expensive, and time consuming. Antimonials, with more than 80 years of history, cause much higher toxicity and associated mortality than the other medicines and should be removed from the treatment recommendations sooner rather than later. On the other hand, it took a long process of more than 10 years after the introduction of MF to build the evidence to demonstrate that the low efficacy observed in children treated with a conventional dose based on weight was associated with underexposure to the drug and that patients weighing ≤30 kg should be treated with an allometric dose. The use of LAB has been extended to rescuing relapses, HIV-VL coinfection and, possibly, PKDL in the Indian subcontinent

due to the donation by Gilead Sciences to WHO. This loss of focus is a risky strategy, because it is clear that the sustainability of a control program cannot depend on a single drug produced by a single supplier and requiring a cold chain. On the other hand, PM is not exempted from side effects, and oral MF is prone to resistance development and has the potential to cause teratogenicity, restricting its use in patients that are pregnant or of child-bearing age. For these reasons, there is a clear need to develop a combination therapy for VL in Africa, but also for the Indian subcontinent once the burden of VL has been reduced.

The assessment of pharmacokinetic-pharmacodynamic relationships between drug exposure and efficacy or safety outcomes is an indispensable component in clinical trials, providing a rationale for planning further studies until satisfactory results are achieved. This is a solid model to follow for the development of orally administered drugs, using potent molecular tools and improved animal models that can better inform the drug posology for humans, and more specifically, children. The future is very promising. For the first time ever, new chemical entities (NCEs) have been designed expressly for leishmaniasis and are not repurposed from other diseases. This rich panoply of oral candidates with a wide range of mechanisms of action will allow for their use in combination to avoid the development of resistance. While the last decade saw interest in leishmaniasis being rescued from oblivion, we can look forward with excitement to the next decade, which will see the establishment of a definitive treatment that meets patient needs.

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Fabiana Alves is the current head of the visceral leishmaniasis clinical program in DND*i*, Geneva, Switzerland. Dr. Alves is a medical doctor who graduated from the University of São Paulo, Brazil, with a residency in pediatrics. A Ph.D. thesis on the molecular epidemiology of malaria in the Amazon region was followed by a postdoctoral position at TDR-WHO. She was a professor of parasitology at the University of São Paulo, coordinated projects at research institutes, and also worked as a project manager for a clinical research organization. She has 17 years of experience in research on tropical diseases, including malaria, leishmaniasis, Chagas disease, and schistosomiasis, working in Latin America, Eastern Africa, and Asia. The last 12 years were mainly dedicated to clinical research for the development of new treatments for neglected diseases, managing clinical trials from phase I through to phase III. With the corresponding author, Dr. Alvas has participated very dynamically in producing the manuscript.

Graeme Bilbe, Ph.D., is the R&D Director for DND*i* and has been responsible for the portfolio at DND*i* that includes leishmaniasis since 2012. Dr. Bilbe received his B.Sc. with honors at Nottingham University with majors in parasitology, neuroscience, and biochemistry and a Ph.D. in Biochemistry from Imperial College University of London. Previously, Dr. Bilbe was Head of Neuroscience Discovery at Novartis Pharma until 2012. Prior to this, he held positions within the company as a researcher in biotechnology, antibody engineering, and molecular biology of diseases, making novel discoveries in the scientific understanding of diseases, including osteoporosis, asthma/allergy, oncology, and neurobiology. His postdoctoral studies were conducted at the Zentrum für Molekulare Biologie at the University of Heidelberg and Imperial College London. He played a major role in the design and review of the manuscript.

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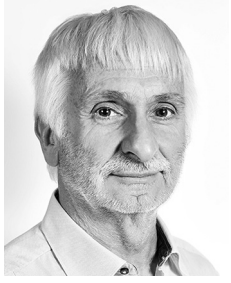
Séverine Blesson is a veterinary doctor whose degree is from Ecole Nationale Vétérinaire d'Alfort (ENVA, France), and she holds an M.Sc. in Immunology from Paris V University. Previously, she worked as a project manager in the department for Research in Resource-Limited Countries at the French National Agency for Research on AIDS and Viral Hepatitis, specifically managing postapproval drug combination clinical trials in Africa and South-East Asia. She is currently Senior Clinical Manager in the Visceral Leishmaniasis Department at DND*i*, Geneva. She has been in charge of the design and follow-up of various clinical trials conducted in Africa in the past 9 years in the field of parasitic diseases, bringing expertise in the field of HIV coinfection. Dr. Blesson provided important feedback in the preparation of the paper.

Vishal Goyal is a medical doctor from India, trained in anesthesia. He is currently working as senior clinical manager in the visceral leishmaniasis (VL) program at DND*i*. He has extensive experience of managing and implementing clinical trials (phases I to IV) and operational research projects in leishmaniasis for the past 8 years in India and Bangladesh. He is certified as a National Accreditation Board for Hospitals & Healthcare Providers (NABH) assessor, carrying out assessments of ethics committees for clinical trials in India. He has presented at numerous national and international conferences. He successfully led and implemented a VL clinical trial in India within public health facilities in collaboration with the national vector-borne disease control program, MSF, State Health Society Bihar, and RMRI (Regional Institute of the Indian Council of Medical Research) that led to national policy change for VL in India in August 2014. For the current paper, he has reviewed the numerous trials conducted by different groups in the Indian subcontinent.

Séverine Monnerat, Ph.D., is a clinical coordinator at DND*i* for the Visceral Leishmaniasis Programme. She obtained her Ph.D. in molecular biology from the University of Lausanne, Switzerland, in 2004, working on *Leishmania* parasites. She then undertook postdoctoral studies on the African trypanosome at the University of Glasgow, Scotland, and the Center for Infectious Disease Research, Seattle, WA, USA, where she also acquired clinical experience at the Malaria Clinical Trial Center. Dr. Monnerat has gained field experience as a volunteer in Southeast Asia and Africa. She worked on implementing a diagnostic test for dengue fever in the Thai-Burma border refugee camps and on malaria in Malawi. Prior to joining DND*i*, she was a scientific officer at FIND, working on human African trypanosomiasis (HAT), Chagas disease, and leishmaniasis, where she gained expertise in diagnostic tools. She has paid special attention to this subject in the current paper, requested by the editor.

Charles Mowbray is the Discovery Director at DND*i*, responsible for advancing new chemical entities into drug development. Dr. Mowbray gained both B.Sc. and Ph.D. degrees in chemistry from the University of Exeter, United Kingdom, and completed postdoctoral fellowships at the University of British Columbia and the University of Nottingham. He then spent 19 years at Pfizer Worldwide Research and Development and was most recently a Research Fellow in Worldwide Medicinal Chemistry at the Sandwich Laboratories, United Kingdom. He worked on a wide range of projects delivering multiple clinical candidates and developed experience as a medicinal chemist, project leader, and personnel manager. Dr. Mowbray is a Fellow of the Royal Society of Chemistry and is an author of over 30 scientific publications and an inventor on 15 patents. He has worked extensively on leishmaniasis over the last 7 years and contributed the section on new chemical entities to this paper.

Gina Muthoni Ouattara, M.D., is a clinical manager at DND*i*. She graduated from the University of Nairobi with a Bachelor of Medicine and Surgery. Prior to joining DND*i*, she worked with the Ministry of Health, ICRH Kenya, MSF France, and University of Nairobi KAVI-Institute of Clinical Research. In the last 12 years, which include her time at DND*i*, her focus has been on infectious diseases and conducting phase I through phase III clinical trials, including HIV drug trials and vaccine trials for HIV and Ebola. She is currently responsible for managing the ongoing phase III visceral leishmaniasis (VL) study in 4 African countries and the PKDL phase II trial in Sudan. For this paper, Dr. Muthoni Ouattara has reviewed and compiled the information for all VL trials conducted in Africa.



Bernard Pécoul, M.D., Doctor *Honoris Causa*, has been the Executive Director of DND*i* since its foundation. Prior to DND*i*, Dr. Pécoul was Director of the MSF Campaign for Access to Essential Medicines from 1998 to 2003, a position he took on after that of Executive Director of MSF-France. While working with MSF, Dr. Pécoul carried out field missions in Africa, Latin America, and Asia. In 1988, he cofounded Epicentre, an MSF-affiliated nongovernmental organization specializing in epidemiology. After obtaining his medical degree at the University of Clermont-Ferrand, France, Dr. Pécoul earned a master's degree in public health at Tulane University, New Orleans, LA, USA. In 2012, he was awarded an honorary Doctor of Laws degree by the University of Dundee, United Kingdom. Bernard Pécoul is a member of the Joint Coordination Board of the Special Programme for Tropical Disease Research (TDR-WHO) and a former board member of UNITAID's Medicines Patent Pool. Dr. Pécoul contributed in the final reading of the manuscript and participated in the conceptualization of most of the trials reported here.

Suman Rijal undertook his medical training from Calcutta Medical College, Kolkata, India, and then trained in internal medicine in the United Kingdom and received an M.R.C.P. (UK) in 1996. He was awarded a Ph.D. in 2006 from the University of Ghent, Belgium. He was a member of the faculty in Internal Medicine and Tropical Diseases at the B. P. Koirala Institute of Health Sciences, Nepal, from 1997 to 2017. In the last 2 decades, he has been actively involved in clinical research and policy development in kala-azar. He has numerous papers on leishmaniasis, with an emphasis on diagnoses and treatment. He is currently the director of DND*i* Delhi. He provided important comments after a deep reading of the first and final versions of the manuscript.

Joelle Rode graduated as a Medical Doctor from Liege University, Belgium, and holds a postgraduate certificate in Tropical Medicine from the Institute of Tropical Medicine of Antwerp. For several years she worked for Médecins Sans Frontières in different contexts in Africa and Brazil. Joelle joined DND*i* Latin America in 2010 and has worked in fundraising, as a clinical trial associate, and as a clinical project coordinator and, since 2015, has been the clinical manager for leishmaniasis in the DND*i* office in Rio de Janeiro, where she coordinates the implementation of clinical trials for leishmaniasis in the region. She reviewed the clinical studies conducted in Latin America and contributed greatly to editing the paper.

Alexandra Solomos, M.Sc., is a Clinical Project Manager at DND*i*, Geneva. She graduated from the University of Geneva with a Master of Sciences in Biology in the field of diabetes. She has 8 years of experience working in clinical research and operations in several therapeutic areas. Ms. Solomos previously worked at Outcome/Quintiles as a Clinical Project Coordinator and Associate Project Manager, coordinating international postapproval and phase IV studies, postauthorisation safety studies (PASS), and drug and disease registries. She joined DND*i* 5 years ago and currently works as a Clinical Project Manager, where she contributes to the implementation and conduct of phase II/III clinical trials for treatment of visceral leishmaniasis in Eastern Africa. Ms. Solomos reviewed all tables for the manuscript.

Nathalie Strub-Wourgaft has been DND*i* Medical Director since 2009 and is an expert in several neglected tropical diseases, including visceral leishmaniasis. In this role, she oversees the regulatory, pharmacovigilance, and quality assurance of all DND*i* trials. She is a key member involved in design, follow-up, and reporting documents and publications for trials related to visceral leishmaniasis which are recorded in this paper. She has a long experience in the private sector. Prior to DND*i*, Dr. Strub-Wourgaft served as Director of Clinical Development at Trophos, and she has over 15 years of clinical development experience, including with Pfizer from 2000 to 2003 and Lundbeck from 1995 to 1999. She also served as Medical Director for a contract research organization (CRO) from 2004 to 2005, as well as for the French office of Aspreva from 2005 to 2008. Dr. Strub-Wourgaft graduated as a Medical Doctor from Necker Hospital, Université René Descartes, in Paris in 1983. Dr. Strub-Wourgaft reviewed the final manuscript and made relevant comments.

Monique Wasunna, M.B.Ch.B., Ph.D., is the Director of the DND*i* Africa Regional Office, based in Nairobi. She is a graduate of the University of Nairobi, School of Medicine, and holds postgraduate qualifications from the London School of Hygiene and Tropical Medicine. She previously was the Acting Director, Kenya Medical Research Institute (KEMRI), and Chief Research Officer and Assistant Director in charge of research at the same institution. She is currently a member of the Kenya National Bioethics Committee, the Kenyatta National Hospital and University of Nairobi Scientific and Research Ethics Committee, and Expert Committee of Clinical Trials of the Pharmacy and Poisons Board, Kenya. For over 25 years, her research interests have been in clinical research in leishmaniasis. Dr. Wasunna has participated in the prioritization of needs, design, follow-up, and reporting of all trials conducted in Eastern Africa reported in this paper and was Principal Investigator in some of the trials cited.

Susan Wells is a research scientist, with a Ph.D. from the Imperial College of Science, Technology and Medicine in London, United Kingdom, for her molecular biology-based research reengineering cytochromes from photosynthetic bacteria. Subsequent to this, she was a postdoctoral research worker in London and then at the Cantonal Hospital in Lausanne, Switzerland. Following a career break, she returned to science, coordinating the tropical disease drug discovery project at Serono in Geneva (now part of Merck KGaA) in collaboration with TDR-WHO and a network of academic institutes. Over the past 7 years at DND*i*, she has managed the communication of scientific and clinical results through publications and presentations on projects ranging from early drug discovery through to late-stage clinical trials. This incorporates diseases such as leishmaniasis, sleeping sickness, Chagas disease, filariasis, mycetoma, and pediatric HIV and includes the key role played in developing open source drug discovery. For this paper, Dr. Wells carried out a deep reading to ensure a coherent discourse.

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Eduard E. Zijlstra, M.Sc., Ph.D., F.R.C.P., F.R.C.Path., trained in internal medicine at the University of Rotterdam, the Netherlands, and further specialized in tropical medicine, infectious diseases, and epidemiology. He worked for Médecins sans Frontières in Sudan in the 1990s in the kala-azar program and has been working in leishmaniasis ever since. He was Professor of Medicine at the College of Medicine in Blantyre, Malawi, from 1999 to 2008. Currently he is Director of the Rotterdam Centre for Tropical Medicine and consultant for the visceral leishmaniasis program in DND*i*. His research interests include various aspects of the practice of medicine in the tropics, with focus on leishmaniasis, mycetoma, HIV/AIDS, and medical education. He is an opinion leader in leishmaniasis, as evidenced by numerous publications, and he is considered a leading expert in post-kala-azar dermal leishmaniasis (PKDL). Dr. Zijlstra contributed to several of the studies reported in the paper and has reviewed various drafts of the paper and added important insights.

Byron Arana, M.D., Ph.D., received his medical training at the Universidad San Carlos of Guatemala, Guatemala, and his doctoral degree in Tropical Medicine from the University of Liverpool, United Kingdom. He has over 20 years' experience working on clinical trials and epidemiological studies, mainly in cutaneous leishmaniasis, onchocerciasis, and diarrheal and respiratory diseases. Dr. Arana is the current head of the cutaneous leishmaniasis clinical program in DND*i* Geneva. Before joining DND*i*, Dr. Arana managed the clinical trials on visceral leishmaniasis that TDR-WHO was supporting in the Indian subcontinent and participated in the development and implementation of strategies in support of the Indian subcontinent's visceral leishmaniasis elimination program. From 2000 to 2008, he served as Co-Director of the Center for Health Studies at Universidad del Valle de Guatemala, Guatemala. Most of his work during recent years has been in looking for new treatment alternatives for cutaneous leishmaniasis. Dr. Arana was key in structuring the paper and made valuable comments on its content at different stages.

Jorge Alvar, M.D., Ph.D., F.R.A.N.M., was graduated as an M.D. from the Complutense University, Madrid (1979), and received the diploma on Tropical Medicine & Parasitology in Hamburg, Germany. He obtained his Ph.D. before spending two years at Cambridge University. Recently, Dr. Alvar has been the head of the Leishmaniasis program at DND*i* since 2013, and from 2018, he has been Senior Scientific Adviser. Previously (2004 to 2012), he headed the Leishmaniasis Control Program at WHO-NTD, having launched an ambitious strategic plan in several countries. Prior to this, he was the Director of the National Centre of Tropical Medicine at the Institute of Health Carlos III (MoH), Madrid. Since 1992, he has been a fellow of the Royal Academy of Medicine (RANM), Spain, and he received its prize in Public Health Research in 2012. He was awarded the Gold Medal of the University of Antioquia, Colombia, and the Research Prize from the Spanish Society of Geography. His interest is focused in epidemiology, chemotherapy, canine leishmaniasis, and AIDS coinfection, with a wide publication record spanning over 30 years. Dr. Alvar conceptualized the paper, wrote, with Fabiana Alves, most of the text, produced the various versions until the final submission, and prepared the responses to the reviewers.