

**Report of a WHO informal consultation
on liposomal amphotericin B
in the treatment of visceral leishmaniasis**

Rome, Italy, 16 April 2005



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Contents

Executive summary	1
Introduction	1
Objectives of the meeting	2
Epidemiology of visceral leishmaniasis	2
Access to antileishmanial treatment	2-3
Pharmacology and pharmacokinetics of liposomal amphotericin B	4
Clinical trials and other experience with liposomal amphotericin B in visceral leishmaniasis	5
Liposomal amphotericin B in HIV-visceral leishmaniasis coinfection	6
Pricing of liposomal amphotericin B	6
Consensus	7
A. Zoonotic visceral leishmaniasis (the Mediterranean Basin, the Middle East and Brazil)	7
B. Anthroponotic visceral leishmaniasis (South Asia, the Horn of Africa)	7
C. HIV-visceral leishmaniasis coinfection	8
D. General	8
References	9-12
Table 1	13
Table 2	14
Table 3	15
Annex. List of participants	16

Executive summary

Liposomal amphotericin B is commonly used to treat visceral leishmaniasis (VL). The World Health Organization (WHO) convened an informal consultation in Rome, Italy, on 16 April 2005 to update the background on and define guidelines for the use of liposomal amphotericin B. In Europe, this formulation is widely used to treat VL in immunocompetent and immunodepressed patients. The VL disease burden is much higher in Africa and Asia than in Europe, and levels of drug availability are low. Public sector agencies can, however, purchase liposomal amphotericin B for distribution through the public sector of developing countries at a preferential price (in July 2007, US\$ 20 per vial of 50 mg). Liposomal amphotericin B (total dose 20 mg/kg) administered in one or two injections has proved to be of high efficacy and low toxicity in immunocompetent VL patients in East Africa, the Mediterranean Basin and Brazil. Results of some controlled trials indicate that a total dose of 10 to 15 mg/kg in South-East Asia may be sufficient to achieve an equally high cure rate. Recommendations on the use of liposomal amphotericin B, either alone or in combination, have been provided for different forms such as zoonotic and anthroponotic VL as well as for patients infected with human immunodeficiency virus (HIV) and visceral leishmaniasis (HIV-VL coinfection).

Introduction

Over the past decade, liposomal amphotericin B has been increasingly used as both a first-line drug to treat patients diagnosed with VL in some endemic regions and as a second-line drug to treat VL patients who fail to benefit from conventional therapy. Liposomal amphotericin B has the highest therapeutic index of existing antileishmanial drugs, a moderately long serum half-life (7 hours) and sustained presence in the tissues for several weeks post-treatment. Historically, the major obstacle to its wider use for VL treatment was its high cost, which has now been reduced to US\$ 20 per vial for public sector agencies wishing to purchase the product for distribution through the public sector of developing countries. WHO policy precludes the recommendation of therapies based on affordability. However, the convergence of recent successful clinical trials in determining the minimum effective total dose and the current preferential pricing provided by the manufacturer for VL patients treated in the public sector means that liposomal amphotericin B may become affordable as a first-line therapy even in resource-constrained settings. Moreover, few new antileishmanial drugs are currently in the development pipeline, and drug resistance is on the rise. Therapy using fixed-dose combinations of drugs is now the standard of care for patients with diseases such as malaria and tuberculosis, and drug resistance is a serious challenge. For these reasons, there is also growing interest in combined regimens of drugs in current use for VL.

Objectives of the Meeting

WHO convened an informal consultation at the Istituto Superiore di Sanità in Rome, Italy, on 16 April 2005. The objectives of the meeting were (i) to discuss current expert knowledge of, and experience with, liposomal amphotericin B in the treatment of VL and (ii) to produce a consensus document with clear guidelines for dosage and clinical use of liposomal amphotericin B for VL. The participants, who represented a wide variety of VL-endemic regions, were experts in specialties ranging from basic research to clinical medicine and access to drugs.

Epidemiology of visceral leishmaniasis

Visceral leishmaniasis, or kala-azar, causes an estimated 500 000 new cases of disease and more than 50 000 deaths every year; 90% of cases occur in just five countries: India, Bangladesh, Brazil, Nepal and Sudan [1]. In South Asia and the Horn of Africa, the predominant mode of transmission is anthroponotic (AVL) [2]. In these areas, humans with kala-azar or post-kala-azar dermal leishmaniasis (PKDL) provide the principal reservoir for ongoing transmission [3, 4], and incomplete or irregular treatment of human VL leads to drug pressure and the rapid development of resistant parasites [5]. In the Mediterranean, the Middle East and Brazil, the disease is zoonotic (ZVL): the domestic dog is the principal reservoir host sustaining transmission to humans [2]. In these regions, most human VL disease occurs in children or immunocompromised adults.

In addition to the distinction between the epidemiology of AVL and that of ZVL, key factors that influence the ability to control VL include the following: poverty and its many effects; poor nutritional status of the population; armed conflict and population movements; ecological changes that alter human contact with the sandfly vector; the prevalence of HIV infection; parasite resistance to antileishmanial drugs; and poor access to health care and antileishmanial drug treatment [6]. In nearly all resource-poor endemic regions, access to antileishmanial drugs is constrained by the economic burden that VL care imposes.

Access to antileishmanial treatment

South Asia has a very high AVL disease burden, characterized by a poorly controlled endemic situation and superimposed large outbreaks such as the one in Bihar State, India, in the early 1990s. The region also suffers from heterogeneous, poorly standardized systems of private health care, where the cost of VL diagnosis and treatment is largely borne by the patient's family. Irregular and incomplete VL treatment courses are common and have led to >60% primary unresponsiveness to pentavalent antimonial drugs (SbV) in northern Bihar [5]. In the southern districts of Bihar, where the rate of SbV resistance is not so high as in the north, SbV is generally available through the government health system and is still in use. Alternative drugs, such as conventional amphotericin B, are available

through only a few nongovernmental organizations and the private sector, severely limiting access to effective antileishmanial drugs in northern Bihar, where the disease burden is highest. Overall, access to antileishmanial drugs in Bihar is rather poor, therefore, and policies to address primary and secondary unresponsiveness to SbV are urgently needed [7].

Substantial levels of SbV resistance are also reported in the districts of Nepal adjacent to northern Bihar [8]. Nepal, however, has public provision of SbV and conventional amphotericin B, and access to antileishmanial drugs is better than in India or Bangladesh. In Bangladesh, the prevalence of SbV resistance still appears to be low, but access to affordable VL treatment is extremely limited [3]. A limited supply of SbV was available through the Government of Bangladesh's health-care system until 2003, when the only licensed manufacturer in the country ceased its production. Since then, access to antileishmanial drugs in Bangladesh has been in crisis, alleviated only temporarily by emergency procurements of SbV through WHO. Neither conventional amphotericin B nor other second-line drugs are provided or sold in VL-endemic districts. In a study conducted in two endemic communities in Bangladesh in 2004, the median direct cost of health care for one VL patient totaled 80% of the yearly per capita income, representing a catastrophic economic burden for affected households.

The Indian government is reviewing policy guidelines that would set specific levels of unresponsiveness to SbV (10–20% in the draft guidelines) as a threshold for changing the first-line drug recommendation [9]. All countries of the South Asian region need urgently to review, update and coordinate existing guidelines for VL treatment. For effective implementation of a rational AVL control policy, health authorities must play a large part in ensuring access to antileishmanial drugs.

In East Africa, nongovernmental organizations provide care free of charge in some areas; in other areas, the private sector provides fee-for-service care. Most VL patients in remote regions, however, find it difficult to access care. In the Horn of Africa, war and population displacements have contributed to explosive VL epidemics with extremely high mortality, often in association with famine and high rates of severe acute malnutrition [10]. Treatment in many parts of this region has been provided by nongovernmental organizations, notably Médecins Sans Frontières (MSF). The current needs include treatment of vulnerable populations, establishment of sentinel surveillance as populations shift again after recent peace accords, and validation of emergency protocols and regimens for their most effective application.

In the ZVL-endemic areas of Brazil and Europe, VL disease burdens are lower than in Asia and Africa, and access to treatment is generally much better [11]. Questions remain, however, regarding optimal treatment regimens for children [12]. In Europe, the incidence of VL as an opportunistic infection in HIV-infected patients has fallen substantially owing to the widespread introduction of highly active antiretroviral therapy (HAART) [13, 14]. Nevertheless, for HIV patients whose immune reconstitution is incomplete, data are insufficient to make firm recommendations on the best regimens for primary treatment and secondary prophylaxis of VL [15, 16].

Pharmacology and pharmacokinetics of liposomal amphotericin B

Liposomal amphotericin B is a lipid formulation of amphotericin B, in which the drug is packaged along with cholesterol and other phospholipids within a small unilamellar liposome [17]. The mechanism of leishmanicidal action is thought to be drug-binding to parasite ergosterol precursors, such as lanosterol, causing disruption of the parasite membrane. The highly specialized liposomal formulation has several characteristics that increase its efficacy against VL while minimizing toxicity. The small size of the liposome (<100 nm) promotes wide distribution and penetration into tissues. The high transition temperature (55 °C, compared with 25 °C for an amphotericin B lipid complex formulation) ensures liposome stability in blood, macrophages and tissues, minimizing release of the drug until it makes contact with the pathogen. The presence in the liposomes of cholesterol, which has an affinity for binding with the drug, may decrease the drug's toxicity by minimizing drug interactions with the mammalian cell membranes. The drug's 10-fold higher affinity for ergosterol and its precursors ensures its antimicrobial efficacy. In mouse models, the LD50 of liposomal amphotericin B is >175 mg/kg, compared with 25–50 mg/kg for other lipid formulations of amphotericin B and 2–3 mg/kg for conventional amphotericin B. Tissue penetration is highest in the liver and spleen, and therapeutic levels persist in these organs several weeks or longer after loading doses of liposomal amphotericin B [18].

Pharmacokinetic studies demonstrate that high initial doses (at least 5 mg/kg, with doses up to 50 mg/kg tested in animals) give better tissue penetration and longer persistence in viscera than frequent low doses, suggesting that initial loading doses may increase efficacy. Loading doses of 60 mg/kg (in 3 doses of 20 mg/kg over a period of 1 week), followed by lower doses, have been shown to be effective for treating fungal infections in animals [19]. In humans, the terminal elimination half-life after repeated administration of liposomal amphotericin B is about 7 hours, with the lowest level in the blood by 24 hours.

Although transient rises in creatinine can occur, acute and chronic toxicity from liposomal amphotericin B is low, even with doses of up to 15 mg/kg [20]. Liposomal amphotericin B requires a fairly reliable cold chain, as exposure to temperatures above 25 °C or below 0 °C will alter characteristics of the liposome. Such changes may increase toxicity or decrease efficacy.

Clinical trials and other experience with liposomal amphotericin B in the treatment of visceral leishmaniasis

Some 13 clinical trials of liposomal amphotericin B for the treatment of VL have been published; most have been open-label, dose-finding studies or randomized open-label comparisons with other antileishmanial drugs (Table 1). In addition, several published case series of patients treated with liposomal amphotericin B describe responses in HIV-VL coinfecting patients and for particular regimens in children [12]. Most of the trials have taken place in India, where at least 10 different regimens have been tested (Table 2). Because of concerns about affordability, one objective of the Indian studies has been to find the lowest total dose with acceptable efficacy. A single dose of 7.5 mg/kg gave a 90% cure rate at 6 months in a fairly large trial (n=203). Total doses of 10 to 20 mg/kg in various dosing schedules gave cure rates >95%, while a single dose of 3.75 mg/kg produced a cure rate of 89% in a limited number of patients (n=28). Indian experience has demonstrated that liposomal amphotericin B caused substantially less toxicity than conventional amphotericin B desoxycholate or amphotericin B lipid complex (ABLC) [28, 32]. Three randomized comparative trials for treatment of fungal infections in neutropenic patients also confirmed significantly lower rates of renal toxicity for liposomal amphotericin B than for either conventional amphotericin B desoxycholate or ABLC [33].

In the Horn of Africa, clinical trial data on liposomal amphotericin B are sparse. However, MSF has extensive clinical experience in treatment of VL in emergency conditions [10, 34]. In Sudan, it has developed a protocol to identify patients at highest risk of death from VL and to triage them to a more intensive treatment plan, including liposomal amphotericin B as initial treatment with a shift to other antileishmanials after clinical improvement, plus aggressive nutritional and medical supportive therapy [34]. These triage protocols, applied on a basis of compassionate use, have substantially reduced case-fatality rates in VL treatment programmes provided by MSF. In Europe, clinical trials have demonstrated 90–98% efficacy with a total liposomal amphotericin B dose of 18–21 mg/kg in immunocompetent patients (Table 2). A variety of regimens are in use.

Liposomal amphotericin B in HIV-visceral leishmaniasis coinfection

There have been no formal randomized clinical trials of liposomal amphotericin B treatment or secondary prophylaxis regimens in HIV-VL coinfecting patients, and only two open-label dose-finding studies (Table 3). Patients with severe immunosuppression have extremely high relapse rates after antileishmanial treatment [24]. A randomized trial of ABLC vs SbV showed comparable efficacy for initial treatment but lower toxicity for ABLC [41]. The efficacy of antimonials and liposomal amphotericin B was comparable in most case series, but the lower rate of toxicity for liposomal amphotericin B has caused most clinicians to consider it as the antileishmanial drug of choice in HIV-VL coinfecting patients.

Secondary prophylaxis with doses of liposomal amphotericin B or other antileishmanials every 2–4 weeks after initial clinical cure of VL is now the standard of care in Europe [15, 16, 42], but data are insufficient to recommend a specific regimen. For some authors, clinical experience to date suggests that discontinuation of secondary antileishmanial prophylaxis can be considered in patients whose CD4 lymphocyte count exceeds 200–350 cells/ μ l in response to HAART, but that prophylaxis should be continued in those with counts below 200 cells/ μ l [16]. Other authors, however, observe that HAART may not be sufficient to control the disease, despite increases in CD4 lymphocyte cell counts and undetectable viral loads, suggesting that secondary prophylaxis should be maintained indefinitely [43, 44].

In Italy, the standard regimen consists of 3 mg/kg on days 1 to 5 and day 10, for a total dose of 18 mg/kg [11]. For imported cases in the USA, the FDA-recommended regimen is 3 mg/kg on days 1 to 5 and on days 14 and 21, for a total dose of 21 mg/kg [35]; in New Zealand, the recommended regimen is 1 to 1.5 mg/kg for 21 days, or 3 mg/kg for 10 days. Published case series and current pediatric practice in southern Europe testify to the efficacy of a total dose of 20 mg/kg; many pediatricians use a regimen of 10 mg/kg/day for 2 consecutive days [12].

Pricing of liposomal Amphotericin B

Pursuant to an agreement between WHO and the manufacturer, liposomal amphotericin B is currently available to public sector agencies for distribution through the public sector of developing countries for the treatment of visceral and mucosal leishmaniasis, at a preferential price of US\$ 20.00 per vial.

Even with the preferential price, a preliminary cost-effectiveness analysis has shown that the recommended regimen, consisting of a total dose of 20 mg/kg, is not competitive with other first-line regimens (SbV, paromomycin, conventional amphotericin B). Nevertheless, the preferential pricing scheme opens perspectives for

liposomal amphotericin B as a recommended second-line treatment and for the inclusion of lower total doses in combination regimens with other antileishmanial drugs.

Consensus

Recommendations for zoonotic visceral leishmaniasis (the Mediterranean Basin, the Middle East and Brazil)

- A total liposomal amphotericin B dose of 20 mg/kg is adequate to treat immunocompetent children and adults in these regions.
- The exact dosing schedule can be flexible (divided into doses of 10 mg/kg on 2 consecutive days or in smaller divided doses), but liposomal amphotericin B pharmacokinetics suggest that the initial dose will provide better tissue levels if at least 5 mg/kg is given.
- The schedule of 10 mg/kg/day on 2 consecutive days needs to be validated in adults with ZVL.
- Veterinary use of liposomal amphotericin B, and other new antileishmanial drugs (miltefosine, paromomycin), should be avoided in order to prevent the development of resistance.

Recommendations for anthroponotic visceral leishmaniasis (South Asia, the Horn of Africa)

- When the rate of unresponsiveness to antimonial drugs exceeds a threshold to be determined in each specific region, policy-makers should strongly consider a shift to an alternative first-line regimen. An Indian expert committee has suggested using thresholds of 10–20% unresponsiveness. Two possible alternative regimens are an amphotericin B formulation, or a combination regimen that does not include antimonial drugs. As monotherapy, liposomal amphotericin B can be given at a total dose of 20 mg/kg in one or two injections in all endemic foci; meanwhile, 10–15 mg/kg for South Asia may be adequate to achieve high cure rates, as is suggested in some limited trials.
- Use of combination antileishmanial drug regimens should be promoted to prevent the development of resistance to existing drugs. Well-conducted trials of specific combinations are urgently needed. An effective regimen would produce an initial parasitological and clinical cure in >95% and a definitive cure at 6 months in >90% of patients.
- With respect to liposomal amphotericin B, the following combinations should be tested: liposomal amphotericin B-miltefosine, liposomal amphotericin B-paromomycin, and (in areas with <10% primary unresponsiveness to SbV) liposomal amphotericin B-SbV.
- If SbV or other monotherapy is used for AVL, the regimen must fulfill WHO guidelines for adequacy (currently >30 days of SbV

at 20 mg/kg/day administered once a day), and all efforts must be made to ensure compliance with complete treatment courses.

- To promote access for all patients, to ensure completeness of treatment and to delay development of drug resistance, the public health community should work in concert with governments and drug companies to provide antileishmanial drugs gratis or at the lowest possible price. To ensure quality of care, and access to care, VL patients should preferably be treated within or in close coordination with an appropriately structured and monitored public health programme.
- The governments of the major VL-endemic countries should facilitate the clinical trials referred to in the above paragraphs and accelerate registration of liposomal amphotericin B and other antileishmanial drugs. Emphasis should be placed on areas where resistance is a problem or HIV-VL coinfection is a major issue.

Recommendations for HIV-visceral leishmaniasis coinfection

- Access to HAART is a high priority for coinfecting patients.
- Multicentre trials of first-line treatment and secondary prophylaxis of VL in HIV-infected patients are needed, and they should include liposomal amphotericin B regimens. Because of stark epidemiological and clinical differences, results from trials in European settings should not be extrapolated to low-income countries, or vice versa.

General recommendations

- An alternative route of liposomal amphotericin B administration (intramuscular, subcutaneous or intrarectal) more easily employed in peripheral health-care settings would be extremely useful. Preclinical work to develop such formulations is encouraged.
- Research is needed to investigate the stability of liposomal amphotericin B in field settings where the cold chain may be suboptimal, and especially in extreme conditions (over 45 °C).
- The current price of liposomal amphotericin B is still too high to use for VL treatment in resource-constrained settings. WHO and others will therefore work with its manufacturer to make it available at a preferential and more affordable price for the public sectors.

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Table 1. **Published clinical trials of liposomal amphotericin B**

Trial	Country	Study design	Total subjects	Reference
1	Brazil	Open-label dose finding	32	[21, 22] ¹
2	Greece	Open-label with historical control	123 ²	[23]
3	India	Open-label dose finding	30	[22, 27] ¹
4	India	Randomized open-label equivalency study, liposomal amphotericin B vs amphotericin B	34	[28]
5	India	Open-label dose finding	91	[29]
6	India	Randomized open-label dose finding	84 ⁶	[30]
7	India	Open-label non-comparison	203	[31]
8	India	Randomized open-label equivalency study, liposomal amphotericin B vs amphotericin B vs amphotericin B lipid complex	153	[32]
9	Italy	Open-label dose finding	31 ³	[24]
10	Italy ⁴	Open-label dose finding	88 ⁵	[25]
11	Italy	Open-label dose finding	106 ²	[26]
12	Kenya	Open-label dose finding	25	[22] ¹
13	Sudan	Open-label dose finding	49	[10]

¹ Multicentre trial in Brazil, India and Kenya.

² All children.

³ Patients who failed to respond to, or relapsed after, treatment with Sb^v.

⁹ 15 immunocompetent children, 5 immunocompetent adults, 11 immunocompromised adults.

¹⁰ 83 cases from Italy, 3 from Brazil, 2 treated in United Kingdom.

¹¹ 56 children, 32 adults.

Trial	Country	Group N	Total dose (mg/kg)	Regimen	Cured (%)	Follow-up (months)	Reported adverse events
13	Sudan	16 16	12 24	3–5 mg/kg days 1, 3, 10 3–5 mg/kg days 1, 2, 6, 8, 10, 13	50 88	Passive Passive	Clinical evaluation only, extravasation in 4 instances, severely ill patients
1	Brazil	15 4	6 10	2 mg/kg day 1, 5, 10 2 mg/kg day 1–4, 10	87 100	6 6	41% fever, 9% chills, 6% respiratory distress, 9% cardiac arrhythmia; treatment stopped in 2
2	Greece	13 41	14 20	1–2 mg/kg days 1–6, 10 10 mg/kg days 1–2	62 98	6 6	7% fever and chills, no discontinuations
3	India	30 10	20 6	4 mg/kg days 1–5 2 mg/kg days 1, 5, 10	90 100	6 6	1 patient with fever, 2 with chills, no discontinuations
4	India	10 17	10 15	2 mg/kg day 1–4, 10 1–2 mg/kg days 1–6, 10 15 mg/kg single dose	100 100	6 6	17% chills (65% Ampho B group), 6% nausea (53% Ampho B group)
5	India	46 45	5 5	5 mg/kg single dose 1 mg/kg days 1–5	91 93	6 6	49% Fever and/or chills, 4% vomiting, 2% back pain, no change in CR
6	India	28 28 28	3.75 7.5 15	0.75 mg/kg days 1–5 1.5 mg/kg days 1–5 3 mg/kg days 1–5	89 93 96	6 6 6	44% infusion-related rigors, 36% fever, 10% back pain, 8% transient rise in CR
7	India	203	7.5	7.5 mg/kg single dose	90	6	10% fever, 3% chills, 4% vomiting, 2% back pain, no renal toxicity
8	India	51	10	2 mg/kg days 1–5	96	6	29% fever and/rigors (98% Ampho B group), no rise in CR (significant rise in Ampho B group)
9	Italy	10	30	3 mg/kg days 1–10	100	12-24	Non-significant rise in BUN, no change in CR, no discontinuations
10	Italy	10 32	21 15	1–1.4 mg/kg days 1–21 3 mg/kg days 1–4, 10	100 91	12-24 12	Mild side-effects, transient rise in BUN and CR, no discontinuations
11	Italy	42 13 16	18 24 15	3 mg/kg days 1–5, 10 4 mg/kg days 1–5, 10 3 mg/kg days 1–3, 5, 10	98 100 75	12 12 12	No adverse events, no change in BUN, CR, electrolytes, LSTs
12	Kenya	66 11 13 5 10 10	18 21 30 6 10 14	3 mg/kg days 1–5, 10 1 mg/kg days 1–21 3 mg/kg days 1–10 2 mg/kg days 1, 5, 10 2 mg/kg day 1–4, 10 1–2 mg/kg days 1–6, 10	98 100 100 20 90 100	12 12 12 6 6 6	Few

Table 2. Efficacy and toxicity of various dosing regimens of liposomal amphotericin B in immunocompetent visceral leishmaniasis patients

Table 3. Published studies of liposomal amphotericin B treatment in HIV-visceral leishmaniasis coinfecting patients

Country	Reference	Study design	Total (mg/kg)	Regimen	N	Initial response	Relapse (%)
Europe ¹	[24]	Open-label dose-finding	29–39	100 mg daily x 21 days	11	Partial clinical response, 9/11 parasite negative at day 21	89 ²
France	[40]	Case series, 2° prophylaxis	60–86 at day 30	2.9–4.1 mg/kg x 5–24 days, then 2.7–3.8 mg/kg q 15d	5	3/5 relapse-free at 13–22 months	40 ³
Greece	[37]	Case series	20, 40	1 mg/kg days 1–7, 1.5 mg/kg days 8–29; 0.75 mg/kg days 1–7; 1.5 mg/kg days 8–17	2	Good clinical response, no relapse at 10–16 months	0
Italy	[39]	Open-label dose finding	40	4 mg/kg days 1–5, 10, 17, 24, 31, 38	10	Partial clinical response, 7/8 parasite negative at day 45	88 ⁴
Spain	[36]	Case series (relapse after Sb ^V)	21, 22.5	1.5 mg/kg x 15 days; 1 mg/kg x 21 days	2	Good clinical response, parasite free at 3–6 months	0
Spain	[38]	Case series	40	4 mg/kg days 1–5, 10, 17, 24, 31, 38	5	Parasites cleared in 80%	40 ⁵

¹ Italy (9), France (1) and Portugal (1).

² Two deaths from other causes, 8 relapses, one cure.

³ Two patients with relapse at 42 and 270 days re-treated with high-dose liposomal amphotericin B followed by prophylaxis with good response in one of the two patients.

⁴ Seven relapses at 2–7 months, two lost to follow-up, one listed as “leishmanin positive”.

⁵ Relapses at 4 and 20 months.

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