

Liposomal Amphotericin B for the Treatment of Visceral Leishmaniasis

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During the past decade, liposomal amphotericin B has been used with increasing frequency to treat visceral leishmaniasis (VL). The World Health Organization convened a workshop to review current knowledge and to develop guidelines for liposomal amphotericin B use for VL. In Europe, liposomal amphotericin B is widely used to treat VL. In Africa and Asia, the VL disease burden is high and drug access is poor; liposomal amphotericin B is available only through preferential pricing for nonprofit groups in East Africa. Clinical trials and experience demonstrate high efficacy and low toxicity for liposomal amphotericin B (total dose, 20 mg/kg) in immunocompetent patients with VL. Combination trials in areas with antileishmanial drug resistance, and treatment and secondary prophylaxis trials in VL–human immunodeficiency virus–coinfected patients, are important to safeguard the current armamentarium and to optimize regimens. The public health community should work to broaden access to preferential liposomal amphotericin B pricing by public sector VL treatment programs.

Over the past decade, liposomal amphotericin B (i.e., AmBisome; Gilead Sciences) has been increasingly used to treat visceral leishmaniasis (VL). Liposomal amphotericin B has the highest therapeutic index of current antileishmanial drugs. The major obstacle to the drug's wider use is its high cost, which is beyond the range of affordability in developing countries with the highest burden of disease. World Health Organization (WHO) policy precludes the recommendation of therapies that are impossible to implement as a result of a lack of affordability. However, recent successful clinical trials to identify the minimum effective total dose and preferential pricing provided by the manufacturer for patients with VL treated in the public sector in East Africa have raised the possibility that liposomal amphotericin B use could become economically feasible for

first-line treatment, even in resource-poor countries. Moreover, there are few new antileishmanial drugs in the pipeline, and drug resistance is on the rise. Combination therapy is now the standard of care for such diseases as malaria and tuberculosis, for which drug resistance is an important challenge. For these reasons, there is growing interest in combination regimens for VL. The WHO convened a consultative meeting at the Istituto Superiore di Sanità (Rome, Italy) on 16 April 2005 to discuss current knowledge of and experience with liposomal amphotericin B for the treatment of VL. The major goal of the workshop was to produce a consensus document with clear guidelines for liposomal amphotericin B dosing and clinical use for VL. Attendees included 15 experts with specialties ranging from basic research to clinical medicine and drug access who represented a wide variety of regions where VL is endemic.

VL causes an estimated 500,000 new cases of disease and 60,000 deaths each year. Ninety percent of cases occur in just 5 countries: India, Bangladesh, Nepal, Sudan, and Brazil [1]. In South Asia and the Horn of Africa, the predominant mode of transmission is anthroponotic [2]. In these areas, humans with kala-azar or post-kala-azar dermal leishmaniasis provide

Received 29 September 2005; accepted 18 January 2006; electronically published 28 August 2006.

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Clinical Infectious Diseases 2006;43:917–24

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1058-4838/2006/4307-0020\$15.00

the major reservoir for ongoing transmission [3, 4], and incomplete or irregular treatment leads to drug pressure and the rapid development of drug-resistant parasites [5]. In the Mediterranean, the Middle East, and Brazil, the disease is zoonotic, with the domestic dog as the most important reservoir host sustaining transmission [2]. In these regions, most human VL disease occurs in children or immunocompromised adults.

In addition to the distinctive epidemiology of anthroponotic VL versus zoonotic VL, key factors that influence the ability to control VL include poverty and its many effects, poor nutritional status of the population, armed conflict and population movements, ecological changes that alter human contact with the sand fly vector, the prevalence of HIV infection, parasite resistance to antileishmanial drugs, and access to health care and antileishmanial drug treatment [6]. In nearly all resource-poor regions of endemicity, access to antileishmanial drugs is constrained by the economic burden that VL care imposes.

ACCESS TO ANTILEISHMANIAL TREATMENT

South Asia has a very high anthroponotic VL disease burden that is 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. 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 a >60% rate of primary unresponsiveness to pentavalent antimonial drugs (Sb^V) in northern Bihar [5]. In southern Bihar, where the rate of Sb^V resistance is lower than in northern districts, Sb^V is generally available through the government health system and is still in use. Alternative drugs, such as conventional amphotericin B, are only available through a few nongovernmental organizations and the private sector, severely limiting effective antileishmanial drug access in northern Bihar, where the disease burden is highest. Overall antileishmanial drug access in Bihar is, therefore, rather poor, and policies to address primary and secondary unresponsiveness to Sb^V are urgently needed [7]. Substantial levels of Sb^V resistance are also reported in the districts of Nepal adjacent to northern Bihar [8]. However, Nepal has a public provision of Sb^V and conventional amphotericin B, and current antileishmanial drug access is better than in India or Bangladesh. In Bangladesh, the prevalence of Sb^V resistance still appears to be low, but access to affordable VL treatment is extremely poor [3, 9]. A limited supply of Sb^V was available through the Bangladesh government health care system until 2003, when the only licensed manufacturer in the country ceased production. Since then, antileishmanial drug access in Bangladesh has been in a state of crisis, alleviated temporarily by emergency procurements of Sb^V through the WHO. Neither conventional amphotericin B nor other second-

line drugs are provided or sold in districts where VL is endemic. In a study in Bangladesh in 2004, the median direct cost of health care for 1 patient with VL totaled 80% of the yearly per capita income, representing a catastrophic economic burden for affected households [9].

The Indian government is currently reviewing guidelines to set specific levels of Sb^V unresponsiveness (10%–20% in the draft guidelines) as a threshold for changing the first-line drug recommendation [10]. Review and coordination of existing VL treatment guidelines are urgently needed for all countries of the South Asian region. To effectively implement a rational anthroponotic VL–control policy, health authorities must play a major role in ensuring access to antileishmanial drugs.

In East Africa, care is provided free of charge by nongovernmental organizations in some areas and through fee-for-service by the private sector in other areas. However, access is difficult for the majority of patients with VL who reside in remote regions. In the Horn of Africa, war and population displacements have contributed to explosive VL epidemics with extremely high mortality rates, often in association with famine and high rates of severe acute malnutrition [11]. Treatment in many areas has been in the hands of nongovernmental organizations, especially Médecins sans Frontières (MSF). Current needs include treatment of vulnerable populations, establishment of sentinel surveillance as populations again shift following recent peace accords, and validation of protocols and regimens used in emergency situations.

In the areas of Europe and Brazil where zoonotic VL is endemic, VL disease burdens are lower than in Asia and Africa, and access to treatment is generally much better [12]. Nevertheless, cost constraints impede liposomal amphotericin B availability for HIV-VL–coinfected patients in Brazil. In addition, questions remain regarding optimal dosing for children [13] and for HIV-infected patients. In Europe, the incidence of VL as an opportunistic infection in HIV-infected patients has decreased substantially as a result of widespread introduction of HAART [14, 15]. However, for HIV-infected patients with incomplete immune reconstitution, data are insufficient to make firm recommendations regarding the best regimens for primary treatment and secondary prophylaxis of VL [16, 17].

PHARMACOLOGY AND PHARMACOKINETICS OF LIPOSOMAL AMPHOTERICIN B

Liposomal amphotericin B is a formulation of amphotericin B in which the drug is packaged with cholesterol and other phospholipids within a small unilamellar liposome. The drug binds to parasite ergosterol precursors, causing disruption of the parasite membrane. The specialized formulation has characteristics that increase efficacy while minimizing toxicity: effective penetration and sustained levels in tissue, especially liver and

spleen; high transition temperature leading to stability in blood, macrophages, and tissues; presence of cholesterol in the liposome, which minimizes drug interaction with mammalian cell membranes and decreases toxicity; and high affinity for ergosterol and its precursors ensuring antimicrobial efficacy [18]. Higher initial doses (≥ 5 mg/kg) provide better penetration and longer tissue persistence than do frequent low doses. Although transient increases in the creatinine level can occur, acute and chronic toxicity is uncommon even with doses up to 15 mg/kg [19]. Exposure to temperatures $>25^{\circ}\text{C}$ or $<0^{\circ}\text{C}$ will alter the liposome's characteristics and may increase toxicity or decrease efficacy.

CLINICAL TRIALS AND OTHER EXPERIENCE WITH LIPOSOMAL AMPHOTERICIN B FOR VL

Thirteen clinical trials of liposomal amphotericin B for the treatment of VL have been published; most were open-label, dose-finding studies or randomized, open-label comparisons with other antileishmanial drugs (table 1). At least 10 different regimens have been tested in India; one objective of these studies has been to find the lowest total dose with acceptable efficacy. A single dose of 7.5 mg/kg yielded a 90% cure rate at 6 months in a fairly large trial ($n = 203$). Total doses of 10–20 mg/kg in various dosing schedules yielded cure rates $>95\%$, whereas a single dose of 3.75 mg/kg led to a cure rate of 89% in a limited number of patients ($n = 28$). Indian experience demonstrated that liposomal amphotericin B caused substantially lower rates of toxicity than conventional amphotericin B desoxycholate or amphotericin B lipid complex (ABLC) [27, 31]. Three randomized, comparative trials for the treatment of fungal infections in neutropenic patients also confirmed significantly lower rates of renal toxicity for liposomal amphotericin B than for conventional amphotericin B desoxycholate or ABLC [32].

In the Horn of Africa, clinical trial data are sparse. However, MSF has extensive clinical experience in VL treatment under emergency conditions [11, 33]. In Sudan, MSF developed a protocol to triage the highest-risk patients to a more intensive regimen, including initial liposomal amphotericin B treatment with a shift to other antileishmanials after improvement, as well as aggressive nutritional and medical supportive therapy [33]. These protocols, applied on a compassionate use basis, have substantially reduced case-fatality rates in MSF VL-treatment programs.

In Europe, clinical trials demonstrated 90%–98% efficacy with a total dose of 18–21 mg/kg in immunocompetent patients (table 2). A variety of regimens are currently in use. In Italy, the standard regimen consists of 3 mg/kg on days 1–5 and 10, for a total dose of 18 mg/kg [12]. For imported cases in the United States, the US Food and Drug Administration recommends 3 mg/kg on days 1–5, 14, and 21, for a total dose of 21

mg/kg [34]. In New Zealand, the recommended regimen is 1–1.5 mg/kg for 21 days or 3 mg/kg for 10 days. Published case series and current pediatric practice in southern Europe suggest good efficacy with a total dose of 20 mg/kg. Many pediatricians currently use a regimen of 10 mg/kg/day for 2 consecutive days [13].

LIPOSOMAL AMPHOTERICIN B IN HIV-VL COINFECTION

There have been no formal, randomized, clinical trials of liposomal amphotericin B treatment or secondary prophylaxis regimens involving HIV-VL-coinfected patients, and there have been only 2 open-label, dose-finding studies (table 2). In patients with severe immunosuppression, relapse rates after antileishmanial treatment are extremely high [23]. A randomized trial of ABLC versus Sb^{V} showed comparable efficacy but lower toxicity for ABLC [40]. The efficacy of Sb^{V} and liposomal amphotericin B were comparable in most case studies, but the lower rate of toxicity for liposomal amphotericin B has caused most clinicians to consider it to be the antileishmanial drug of choice in VL-HIV-coinfected 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 [16, 17, 41], 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 counts increase to >200 – 350 cells/ μL in response to HAART, but that prophylaxis should be continued in those with counts <200 cells/ μL [17]. However, other authors observe that HAART is not sufficient to control the disease, despite increases in the CD4^+ lymphocyte count and undetectable viral loads, suggesting that secondary prophylaxis should be maintained indefinitely [42, 43].

LIPOSOMAL AMPHOTERICIN B PRICING

In 1992, an agreement between the WHO and Vestar led to preferential pricing for liposomal amphotericin B for patients with VL of \$50 (in US dollars) per vial; a negotiation in 2004 led to the even more reduced price of €22.30 per vial. This price is valid for liposomal amphotericin B for patients with VL who are treated by not-for-profit institutions in East Africa, but not for treatment of other diseases or for patients with VL in other regions. An extension of this preferential pricing to include patients with VL in India, Bangladesh, Nepal, and Brazil could improve access to effective treatment and save many lives. Even with preferential pricing, liposomal amphotericin B (total dose, 20 mg/kg) is not as cost-effective as other first-line regimens (i.e., Sb^{V} , paromomycin, and conventional amphotericin B). Nevertheless, preferential pricing opens the prospect of liposomal amphotericin B as second-line treatment and for in-

Table 1. Efficacy and toxicity of various dosing regimens of liposomal amphotericin B (LAmB) in immunocompetent patients with visceral leishmaniasis.

Country	Reference(s)	Study design	No. of subjects		Total AmB dose, mg/kg	LAmB regimen	Percentage of cured subjects	Follow-up duration, months	Reported adverse events ^a
			Total	Per group					
Brazil	[20, 21] ^b	Open-label, dose-finding	32	15	6	2 mg/kg on day 1, 5, and 10	87	6	Fever, 41%; chills, 9%; respiratory distress, 6%; cardiac arrhythmia, 9%; treatment was stopped for 2 subjects
				4	10	2 mg/kg on days 1–4 and 10	100	6	...
				13	14	1–2 mg/kg on days 1–6 and 10	62	6	...
Greece	[22]	Open-label with historical control	123 ^c	41	20	10 mg/kg on days 1–2	98	6	Fever and chills, 7%; no discontinuations of treatment
				30	20	4 mg/kg on days 1–5	90	6	...
Italy	[23]	Open-label, dose-finding	31 ^d	10	30	3 mg/kg on days 1–10	100	12–24	Nonsignificant increase in BUN level; no change in creatinine level; no discontinuations of treatment
				10	21	1–1.4 mg/kg on days 1–21	100	12–24	...
Italy ^e	[24]	Open-label, dose-finding	88 ^f	32	15	3 mg/kg on days 1–4 and 10	91	12	Mild adverse effects; transient increase in BUN and creatinine levels; no discontinuations of treatment
				42	18	3 mg/kg on days 1–5 and 10	98	12	...
				13	24	4 mg/kg on days 1–5 and 10	100	12	...
Italy	[25]	Open-label, dose-finding	106 ^c	16	15	3 mg/kg on days 1–3, 5, and 10	75	12	No adverse events, no change in levels of BUN, creatinine, electrolytes, or liver enzymes
				66	18	3 mg/kg on days 1–5 and 10	98	12	...
				11	21	1 mg/kg on days 1–21	100	12	...
				13	30	3 mg/kg on days 1–10	100	12	...
India	[21, 26] ^b	Open-label, dose-finding	30	10	6	2 mg/kg on days 1, 5, and 10	100	6	One patient had fever, and 2 had chills; no discontinuations of treatment
				10	10	2 mg/kg on days 1–4 and 10	100	6	...
				10	14	1–2 mg/kg on days 1–6 and 10	100	6	...

India	[27]	Randomized, open-label equivalency	34	17	15	Single 15-mg/kg dose	100	6	Chills, 17% (65% of subjects in ConAmB group); nausea, 6% (53% of subjects in ConAmB group)
India	[28]	Open-label, dose-finding	91	46	5	Single 5-mg/kg dose	91	6	Fever and/or chills, 49%; vomiting, 4%; back pain, 2%; no change in creati- nine level
India	[29]	Randomized, open-label, dose- finding	84 ^g	45	5	1 mg/kg on days 1–5	93	6	...
				28	3.75	0.75 mg/kg on days 1–5	89	6	Infusion-related rigors, 44%; fever, 36%; back pain, 10%; transient increase in creatinine level, 8%
				28	7.5	1.5 mg/kg on days 1–5	93	6	...
				28	15	3 mg/kg on days 1–5	96	6	...
India	[30]	Open-label noncomparison	203	203	7.5	Single 7.5-mg/kg dose	90	6	Fever, 10%; chills, 3%; vomiting, 4%; back pain, 2%; no renal toxicity
India	[31]	Randomized, open-label equivalency	153	51	10	2 mg/kg on days 1–5	96	6	Fever, 29%; rigors in 98% of subjects in ConAmB group; no increase in creati- nine level (but a significant increase in the ConAmB group)
Kenya	[21] ^b	Open-label, dose-finding	25	5	6	2 mg/kg on days 1, 5, and 10	20	6	Few
				10	10	2 mg/kg on days 1–4 and 10	90	6	...
				10	14	1–2 mg/kg on days 1–6 and 10	100	6	...
Sudan	[11]	Open-label, dose-finding	49	16	12	3–5 mg/kg on days 1, 3, and 10	50	Passive	Clinical evaluation only; 4 instances of extravasation; patients in study were severely ill
				16	24	3–5 mg/kg on days 1, 2, 6, 8, 10, and 13	88	Passive	...

NOTE. BUN, blood urea nitrogen; ConAmB, conventional AmB desoxycholate.

^a Incidence of adverse events in the LAmB group (versus comparison group, where appropriate).

^b Multicenter trial in Brazil, India, and Kenya.

^c All subjects were children.

^d Study population included 15 immunocompetent children, 5 immunocompetent adults, and 11 immunocompromised adults.

^e Study included 83 cases from Italy, 3 cases from Brazil, and 2 cases treated in the United Kingdom.

^f Study population included 56 children and 32 adults.

^g Patients who did not respond to or relapsed after treatment with pentavalent antimonial drugs.

Table 2. Findings of published studies of liposomal amphotericin B (LAmB) treatment in HIV-visceral leishmaniasis-coinfected patients.

Country	Reference	Study design	No. of subjects	Total LAmB dose, mg/kg	Regimen	Initial response	Relapse rate, %
Spain	[35]	Case series (relapse after Sb ^v treatment)	2	22.5	1.5 mg/kg per day for 15 days	Good clinical response, parasite free at 3–6 months	0
				21	1 mg/kg per day for 21 days
Greece	[36]	Case series	2	40	1 mg/kg per day for days 1–7 and 1.5 mg/kg per day for days 8–29	Good clinical response; no relapse at 10–16 months	0
				20	0.75 mg/kg per day for days 1–7 and 1.5 mg/kg per day for days 8–17
Spain	[37]	Case series	5	40	4 mg/kg per day for days 1–5, 10, 17, 31, and 38	Parasites cleared in 80% of subjects	40 ^a
Europe ^b	[23]	Open-label, dose-finding	11	29–39	100 mg per day for 21 days	Partial clinical response in 9 of 11 subjects; negative for parasites at day 21	89 ^c
Italy	[38]	Open-label, dose-finding	10	40	4 mg/kg per day for days 1–5, 10, 17, 31, and 38	Partial clinical response in 7 of 8 subjects; negative for parasites at day 45	88 ^d
France	[39]	Case series, secondary prophylaxis	5	60–86 by day 30	2.9–4.1 mg/kg per day for 5–24 days, followed by 2.7–3.8 mg/kg every 15 days to prevent relapse	3 of 5 subjects were relapse free at months 13–22	40 ^e

NOTE. Sb^v, pentavalent antimonial drugs.

^a Relapses at 4 and 20 months.

^b Nine subjects from Italy, 1 from France, and 1 from Portugal.

^c Two deaths due to other causes, 8 relapses, and 1 cure.

^d Seven subjects experienced relapses at 2–7 months, 2 were lost to follow-up, and 1 was listed as “leishmanina positive.”

^e Two patients had relapse at 42 and 270 days and were re-treated with high-dose liposomal LAmB followed by prophylaxis, with good response in 1 of the 2 patients.

clusion of lower total doses in combination regimens. To file for an extension of the preferential pricing scheme, figures detailing the projected annual uptake will be compiled under the leadership of the WHO.

RECOMMENDATIONS

Zoonotic VL (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 zoonotic VL.
 - Veterinary use of liposomal amphotericin B and other new antileishmanial drugs (e.g., miltefosine and paromomycin) should be avoided to prevent the development of resistance.
- Anthroponotic VL (South Asia and the Horn of Africa)
- When 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 (for example, liposomal amphotericin B at a total dose of 20 mg/kg) or a combination regimen that does not include Sb^v.

- 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. A regimen would be considered effective if it produces an initial parasitologic and clinical cure in $\geq 95\%$ of patients and a definitive cure at 6 months in $\geq 90\%$ of patients.
- With respect to liposomal amphotericin B, the following combinations should be tested: liposomal amphotericin B plus miltefosine, liposomal amphotericin B plus paromomycin, and (in areas with $<10\%$ primary unresponsiveness to Sb^V) liposomal amphotericin B plus Sb^V .
- If Sb^V or other monotherapy is used for anthroponotic VL, it is imperative that the regimen fulfills WHO guidelines for adequacy (currently, ≥ 30 days of Sb^V at 20 mg/kg/day administered once per day) and that all efforts are 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 and access to care, patients with VL should preferably be treated within or in close coordination with an appropriately structured and monitored public health program.
- The governments of the countries where VL endemicity is major should facilitate the clinical trials outlined above and accelerate registration of liposomal amphotericin B and other antileishmanial drugs. Emphasis should be placed on areas where resistance is a problem or where HIV-*Leishmania* coinfection is a major issue.

HIV-VL Coinfection

- Access to HAART is high priority for HIV-VL-coinfected patients.
- Multicenter trials of first-line treatment and secondary prophylaxis of VL in HIV-infected patients are needed, and liposomal amphotericin B regimens should be included in these trials. Because of stark epidemiologic and clinical differences, results from trials in European settings should not be extrapolated to apply to low-income countries, and vice versa.

General

- An alternative route of liposomal amphotericin B administration that is more easily employed in peripheral health care settings (intramuscular, subcutaneous, or intrarectal) 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 to investigate it especially in extreme

conditions (temperatures $>45^\circ\text{C}$).

- The current price of liposomal amphotericin B is prohibitively high for VL treatment in resource-poor countries. Therefore, the WHO and others will work with its manufacturer to make it available at a preferential and more affordable price for the public sectors in India, Bangladesh, and Nepal and for programs that treat HIV-VL-coinfected patients in Brazil.

Acknowledgments

We thank the Istituto Superiore di Sanità, Rome for kindly providing the meeting facilities.

Financial support. The consultative meeting on which this article is based was supported by Communicable Disease Control, Prevention and Eradication, WHO (Geneva, Switzerland), and the Italian Cooperation.

Potential conflicts of interest. J.A.-M. has served as consultant to, is a member of the speakers' bureau of, and has received research grants from manufacturers of liposomal amphotericin B (Gilead Sciences and Fujisawa Healthcare [now Astella]). J.B. has served as a consultant to Gilead in relation to antiretroviral compounds. R.N.D. has received research funding from the manufacturers of liposomal amphotericin B (Gilead Sciences) and has acted as a consultant for a nonprofit company developing paromomycin (Institute of OneWorld Health). S.S. has received support for clinical trials and presentation of data at scientific meetings from the manufacturers of liposomal amphotericin B (Nextar Pharmaceuticals [now Gilead Sciences]). J.A. received institutional support for clinical trials from the manufacturers of amphotericin B lipid complex (PENSA, Grupo Esteve) and is a member of the scientific board of *Microbosome*, a journal funded by Vestar. All other authors: no conflicts.

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