

# U.S. Food and Drug Administration Approval of AmBisome (Liposomal Amphotericin B) for Treatment of Visceral Leishmaniasis

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In August 1997, AmBisome (liposomal amphotericin B, Nexstar, San Dimas, CA) was the first drug approved for the treatment of visceral leishmaniasis by the U.S. Food and Drug Administration. The growing recognition of emerging and reemerging infections warrants that safe and effective agents to treat such infections be readily available in the United States. The following discussion of the data submitted in support of the New Drug Application for AmBisome for the treatment of visceral leishmaniasis shows the breadth of data from clinical trials that can be appropriate to support approval for drugs to treat tropical diseases.

In August 1997, AmBisome (liposomal amphotericin B; Nexstar, San Dimas, CA) was approved by the U.S. Food and Drug Administration (FDA) for the treatment of visceral leishmaniasis (VL). This is the first drug approved for this indication in the United States. As awareness of the emergence and reemergence of the more exotic, traditionally tropical diseases grows, so does the need for well-documented evidence of the safety and efficacy of the drugs used to treat these infections. The Division of Special Pathogens and Immunologic Drug Products, FDA, which has regulatory responsibility for all antiparasitic drugs, advises early dialogue in the development process of drugs for the treatment of tropical diseases. Such discussion can facilitate the submission and review of applications. The subsequent approval process can provide greater access to drugs for the treatment of these infections.

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See editorial response by Berman on pages 49–51.

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Herein, the clinical and microbiological data submitted to support the approval of AmBisome for the treatment of VL are reviewed. An example of a drug approval that used an historical control and data from studies conducted outside the United States is provided. The discussion illustrates the breadth of data from clinical trials that can be utilized to support drug approval and serves as a point from which to offer suggestions about future trial design.

## Historical Perspective

In 1903, Leishman [1] reported an account of Dumdum fever, a febrile illness encountered in British soldiers serving in

the Indian cantonment of Dumdum near Calcutta. Leishman, a pathologist, noted that these patients were distinguished from other patients with fever by profound cachexia and unusually large spleens. The microscopic examination of the postmortem spleen from one such patient revealed many small round bodies. He speculated that these forms were trypanosomes, the description of which had recently been made by Bruce. Leishman suggested that patients with kala-azar and sleeping sickness also be studied after death for the presence of such round bodies. It is noteworthy that kala-azar, the disease entity described by the Hindi expression for darkening of the skin, was a well-described clinical syndrome at the time of Leishman's report. Kala-azar only later came to be known as VL, the infection of major organs caused by parasites of the genus that was ultimately named *Leishmania*.

Recent developments in human experience with this genus of parasite have made it a topic of growing interest, which was perhaps most highly publicized by the diagnosis of viscerotropic leishmaniasis in veterans who served in the Persian Gulf in 1991 [2]. In the 1980s, VL became recognized as an opportunistic infection in HIV-positive patients in southern Europe. To date, >1,000 such cases have been reported [3]. Epidemics of visceral disease have been seen in the Sudan and Brazil in the past decade [4]. A growing number of cases in which there was resistance to the traditional first-line therapy, pentavalent antimonials, has been documented in India [5].

## Clinical Considerations

### Diagnosis

Since the association made by Leishman in the early part of this century, VL has been diagnosed by microscopic visualization of the amastigote stage of the parasite in infected tissues, usually spleen or bone marrow. Culture of the parasite from infected tissue can increase the diagnostic yield. Although skin testing, antibody detection, and, most recently, PCR analysis have been evaluated for their usefulness in the diagnosis of VL, each of these diagnostic methods has limitations. Micros-

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**Table 1.** *Leishmania* species causing visceral disease.

Species	Geographic distribution
<i>Leishmania infantum</i>	Mediterranean basin, including southern Europe and North Africa, Iran, and central Asia
<i>Leishmania donovani</i>	India and East Africa
<i>Leishmania chagasi</i>	South and Central America

NOTE. The species listed are classically associated with visceral leishmaniasis. Cases of visceral disease caused by *Leishmania tropica* and *Leishmania mexicana* have also been documented.

copy, with or without culture, remains highly sensitive and specific and is the diagnostic method of choice.

### Epidemiology

The genus *Leishmania* includes several species that are pathogenic to humans. The infections caused by this genus are best viewed as a complex of diseases. Clinical manifestations of infection are generally characterized as cutaneous leishmaniasis or VL. There are three species of the genus *Leishmania* associated with most visceral disease in humans. Each of these species is associated with a specific geographic distribution as described in table 1. Occasionally, species that are associated with cutaneous syndromes such as *Leishmania tropica* and *Leishmania major* are isolated from patients with VL. Less than 20% of infections with *Leishmania donovani*, *Leishmania infantum*, or *Leishmania chagasi* progress to clinically apparent visceral disease [4].

Response to infection is determined in part by the immune status of the host. Although the presentation of VL is fairly uniform throughout the world, response to treatment is not. Reports of resistance to standard treatments such as antimonials are increasing in parts of Europe, India, and East Africa [4, 6, 7]. Because of these variations in tissue tropism, host response, and geography, it is important to carefully define any population of patients with VL who are to be studied.

### Treatment

Early treatment for VL was attempted with the trivalent antimonials. The less toxic pentavalent antimonials were introduced in the 1920s. This class of drugs, which includes sodium stibogluconate (Pentostam; Burroughs Wellcome, Research Triangle Park, NC) and meglumine antimonate (Glucantime; Rhône-Poulenc, Antony Cedex, France), has been the mainstay of treatment for VL since its introduction. As experience with these drugs has grown, so has the incidence of treatment failures. The 1980s and 1990s have seen the recommended dosage of pentavalent antimony increase from 10 to 20 mg/(kg · d) and the length of treatment extend from 20 to 28 days to periods as long as 60 days in regions with high rates of clinical failures.

Some investigators have speculated that these increases are because of the development of relative resistance to antimonials [8, 9]. The coinfection of patients with HIV disease and VL has introduced another population of patients not cured with standard doses of antimonials.

Other drugs shown to have efficacy in treating *Leishmania* infections include aminosidine (paromomycin), pentamidine, and amphotericin B. Indeed, amphotericin B and its lipid formulations have assumed a growing importance in the treatment of VL. One of the few reported trials that prospectively compared pentavalent antimony with amphotericin B was undertaken in a region of India with a high rate of clinical failure among patients treated with antimonials [10]. This study of previously untreated patients demonstrated a success rate of 100% for those treated with amphotericin B compared with 62.5% for those treated with intramuscular stibogluconate. The declining efficacy of antimonials in some areas of the world and treatment options of a given locale have evolved such that amphotericin B and its lipid complexes have come to be viewed as first-line agents along with pentavalent antimonials [11].

Amphotericin B is thought to act by binding with parasite precursors of ergosterol in preference to host cholesterol, thereby interrupting parasite cell wall synthesis. Although this drug has shown excellent in vitro activity against *Leishmania*, the toxicity of amphotericin B deoxycholate has limited its clinical use. Preparations of amphotericin B lipid complexes have been thought to have potential utility in the treatment of VL because of excellent distribution into the intracellular compartment, where parasites reside in the human host. The first clinical case report of successful treatment of VL with AmBisome was reported in 1991 [12]. Subsequent reported studies of other preparations of amphotericin B lipid complexes for the treatment of VL also demonstrated efficacy. Two Amphocil (Sequus Pharmaceuticals, Menlo Park, CA) regimens evaluated in a small series in Brazil had efficacy rates of 90%–100% for previously untreated patients [13]. Low-dose therapy with Abelcet (Liposome Company, Princeton, NJ) for VL patients in India who had had poor responses to pentavalent antimony was studied; the long-term efficacy rate was 84%–100% [14].

### Methods

#### Submission of New Drug Application (NDA)

The clinical data that supported the approval of AmBisome for the treatment of VL included a study that summarized the results of four clinical trials in the medical literature [15–18] and case reports for all patients included in three of these studies [15–17]. All of the clinical trials were conducted outside the United States. Three of the trials [15–17] included immunocompetent and immunosuppressed patients seen in referral centers in Europe and Brazil. The study populations described in these three reports were pooled and analyzed by the

**Table 2.** AmBisome for the treatment of visceral leishmaniasis: evaluability and efficacy by treatment regimen.

Treatment group, cohort (regimen)	Drug sponsor analysis				FDA analysis		
	Total dose (mg/kg)	No. of patients evaluable	No. with success at EOT*/no. of evaluable patients (%)	No. relapse-free at follow-up <sup>†</sup> /no. of evaluable patients (%)	No. of patients evaluable	No. with success at EOT*/no. of evaluable patients (%)	No. relapse-free at follow-up <sup>‡</sup> /no. of evaluable patients (%)
<b>Immunocompetent patients</b>							
I (1 mg/[kg · d] or 100 mg/d for 21 d)	21–29	10	10/10 (100)	10/10 (100)	5	5/5 (100)	5/5 (100)
II (3 mg/[kg · d] for 10 d)	30	10	10/10 (100)	10/10 (100)	10	10/10 (100)	10/10 (100)
III (4 mg/[kg · d] on d 1–5 and 10)	24	13	13/13 (100)	10/10 (100)	10	10/10 (100)	10/10 (100)
IV (3 mg/[kg · d] on d 1–5 and 10)	18	42	42/42 (100)	40/41 (97.6)	33	33/33 (100)	32/33 (97.0)
V (3 mg/[kg · d] on d 1–4 and 10)	15	32	31/32 (96.9)	29/31 (93.5)	28	27/28 (96.4)	25/28 (89.3)
VI (3 mg/[kg · d] on d 1–3 and 10)	12	1	1/1 (100)	1/1 (100)	1	1/1 (100)	1/1 (100)
Total		108	107/108 (99.1) (97.3–100.0) <sup>§</sup>	100/103 (97.1) (93.8–100.0) <sup>§</sup>	87	86/87 (98.9) (96.6–100.0) <sup>§</sup>	83/87 (95.4) (91.0–99.8) <sup>§</sup>
<b>Immunosuppressed patients</b>							
VII (100 mg/d for 21 d)	29.0–38.9				10	10/10 (100)	2/10 (20.0)
VIII (4 mg/[kg · d] on d 1–5, 10, 17, 24, 31, and 38)	40				9	8/9 (88.9)	0/7
Total					19	18/19 (94.7)	2/17 (11.8)

NOTE. EOT = end of therapy; FDA = U.S. Food and Drug Administration. AmBisome is manufactured by Nexstar, San Dimas, CA. The drug sponsor definition of the success rate at follow-up was the no. of relapse-free patients at follow-up/no. with parasitological clearance at EOT; the FDA definition of the success rate at follow-up was the no. of patients with parasitological clearance at EOT who remained relapse-free/total no. of evaluable patients.

\* Parasitological clearance.

<sup>†</sup> Clinical cure.

<sup>‡</sup> Overall success.

<sup>§</sup> 95% CI.

drug sponsor according to immune status and to the dosing regimen that they received. The dosing regimens used for the various cohorts of patients treated in these referral centers are summarized in table 2. The fourth report [18] described a series of patients treated under field conditions in the Sudan. Because these patients had more advanced disease, received different dosing regimens of AmBisome, and had less consistent follow-up than those in the referral centers, the data from the Sudan were not considered central to the application by the FDA. The following discussion of the data supporting the FDA approval of AmBisome for the treatment of VL is based on the results of treatment of patients in Europe and Brazil.

It should be noted that treatment of VL was one of several indications being requested in this NDA; therefore, the safety database available was larger than the population of patients with VL who were studied. The other indications for which AmBisome was approved were empirical therapy for febrile neutropenia and the treatment of aspergillus, candida, and/or cryptococcus infection refractory to traditional amphotericin B or for patients with renal impairment. For VL, the efficacy

database included 129 patients. The safety database for the entire NDA included >1,500 patients.

### Design of Clinical Trials

Spleen, bone marrow, or liver aspirates were obtained from patients with clinically suspected VL. For patients to be included in the study, amastigotes had to be visualized in or cultured from the aspirate. During treatment and follow-up, weight, temperature, spleen size, routine serum chemistry measurements, hematologic findings, and erythrocyte sedimentation rate were recorded at regular intervals. Another aspirate was obtained at the end of therapy for microscopy and culture. The parasite count in the spleen or bone marrow aspirate was quantified by the method of Chulay and Bryceson [19]; this parasitological end point was considered the test of cure. Follow-up visits were scheduled at 1, 3, 6, and 12 months after treatment; at these visits, patients were assessed by clinical examination and repeated laboratory tests. Parasitological stud-

ies were done if there were any clinical or laboratory findings suggestive of relapse.

AmBisome therapy for immunocompetent patients infected in southern Europe or Brazil was studied. Approximately one-third of these patients' isolates were identified to the species level; all isolates from patients in southern Europe that were identified to the species level were *L. infantum*. Clinical trials began with a dose that was thought adequate to cure. Subsequent cohorts were then treated with progressively lower doses or shorter courses of therapy in an attempt to establish the lower limit of efficacy. In total, there were six cohorts of immunocompetent patients studied (table 2, cohorts I–VI). The clinical trials including immunosuppressed patients enrolled patients infected in the Mediterranean basin, including Europe and North Africa; because of the high relapse rate among the first cohort, a second, more intensively treated cohort was studied (table 2, cohorts VII and VIII).

### FDA Approach to Review

The combination of the requirement for parasitological identification, the large number of patients studied, and the length of time that patients were followed up after treatment made these studies unusual in the medical literature on drug treatment of VL. At the time of the review of the NDA for AmBisome, there were no drugs approved for use in the United States for the treatment of VL. It was not possible to study the efficacy of AmBisome in a controlled trial with an approved active comparator. Untreated, the disease is regarded as ultimately fatal; therefore, a historical control was invoked. The fatal outcome of untreated disease was considered the initial basis from which to determine drug effect. For purposes of approval, it was then necessary to establish the efficacy of the drug(s) regarded as the standard of care in the treatment of VL; the efficacy of AmBisome would then be compared with this standard. A review of the literature and personal communications with experts suggested that the primary response rate associated with an effective drug should be 90%–95%.

## Results

### Evaluability Criteria

A total of 108 immunocompetent patients and 21 immunosuppressed patients were enrolled in the study. All patients were considered evaluable by the drug sponsor. Of the immunocompetent patients, ~65% were children, 56% were male, and 7% had had prior therapy for VL. Of the immunosuppressed patients, 81% were HIV-positive and 62% had had prior therapy for VL. A more exclusive analysis of evaluability was performed by the FDA. All case reports for patients in European and Brazilian centers were reviewed. Patients who were deemed evaluable by the FDA were those who were infected in the Mediterranean basin, had no prior treatment

with antileishmanial drugs or had treatment  $\geq 1$  month before enrollment in the present trial, had a record of undergoing a repeated parasitological study at end of therapy, and were followed up  $\geq 6$  months after treatment.

### Efficacy

The analysis of efficacy performed by the drug sponsor used two separate end points: acute clearance of parasites as determined by microscopy and/or culture of spleen or bone marrow aspirates at the end of therapy and overall success, which included those patients with clearance of parasites at the end of therapy who remained relapse-free in the follow-up period 6–12 months after completion of therapy. Failures were defined by parasites in repeated tissue aspirates at the end of therapy and relapses following acute clearance of parasites. The following discussion of the analysis of the clinical trial data for immunocompetent hosts distinguishes between the drug sponsor and the FDA analyses of these populations. Both analyses are presented in table 2.

*Immunocompetent patients—drug sponsor analysis.* The efficacy rates calculated in the drug sponsor analysis at both the end of therapy and follow-up are presented in table 2; the 95% confidence intervals around these point estimates were well within the efficacy rate range of 90%–95% that is expected for an effective antileishmanial drug. The drug sponsor defined the success rate as the number of patients who remained relapse-free during  $\geq 6$  months of follow-up divided by the number of patients with parasitological clearance at the end of therapy.

*Immunocompetent patients—FDA analysis.* The use of more exclusive evaluability criteria determined by the FDA (see above) identified 87 immunocompetent patients who were clinically and parasitologically evaluable. In the FDA analysis, failures at 21 days were carried forward and included in the calculation of the overall success rate. By this scheme, the success rate was defined as the number of patients cured at the end of therapy who did not relapse divided by the total number of evaluable patients. The overall success rate is a more stringent measurement of efficacy. On the basis of these criteria, efficacy rates associated with AmBisome as treatment of VL in immunocompetent patients remained in the range expected for an effective antileishmanial agent at both the end of therapy and follow-up (table 2).

The total dose approved for the treatment of VL is 21 mg/kg given on 7 days over a 21-day period. At the end of therapy, the efficacy rate associated with AmBisome as treatment of VL in patients who received  $\geq 21$  mg/kg (cohorts I, II, and III) was 100% (33 of 33) in the drug sponsor analysis and 100% (25 of 25) in the more stringent analysis performed by the FDA. The efficacy rate for these patients at follow-up was 100% (30 of 30) and 100% (25 of 25), respectively. The patients in cohort IV received a total dose of 18 mg/kg. The overall success rates for cohort IV at follow-up were 97.6%

(40 of 41) in the sponsor analysis and 97.0% (32 of 33) in the FDA analysis. Efficacy was maintained for patients receiving less than the recommended dose in both analyses. Such efficacy rates were viewed as supportive of the dosing regimen that was approved.

**Immunosuppressed patients—drug sponsor and FDA results.** The response to treatment in the first cohort of immunosuppressed patients (cohort VII) was characterized by a higher rate of relapse than seen among the immunocompetent patients. Because of this finding, a second cohort of immunosuppressed patients was treated with a more intensive regimen (cohort VIII). The relapse rate remained high among these patients (table 2). This finding was noted in both the drug sponsor analysis and the FDA analysis of the results. Although some level of clinical response and clearance of parasites was noted for most immunosuppressed patients treated with the above-mentioned regimens, the high relapse rates of 80% and 100% are remarkable.

## Safety

Nine reported treatment-emergent adverse events (6.9%) occurred in the 129 VL patients treated in Europe or Brazil. Assessment of relationship to the study drug was not made in all cases, but no patient's treatment regimen was changed because of a clinical adverse event or because of abnormal laboratory findings. One episode of mild arterial hypotension was reported after the first dose. Rashes, nausea and/or vomiting, and headache were observed in three or fewer patients. There were no episodes of phlebitis associated with infusion. Review of the larger safety database of >1,500 patients provided some comparative safety information on AmBisome and amphotericin B. In some subgroups, AmBisome was associated with lower levels of nephrotoxicity and fewer episodes of hypokalemia than was amphotericin B.

## Discussion

### Results of AmBisome Clinical Trials

The submitted studies demonstrated the efficacy of AmBisome in the treatment of VL in immunocompetent patients (both adult and pediatric) in the Mediterranean basin who had documented or presumed *L. infantum* infection. Data supporting the clinical efficacy of AmBisome as treatment of VL caused by other species and from other geographic foci were not provided by this NDA. It is noteworthy that in vitro data do demonstrate activity of AmBisome against *L. donovani* and that the medical literature includes reports of the therapeutic use of AmBisome for VL patients in India, where the infection is generally caused by *L. donovani* [20].

The markedly higher relapse rate noted among the immunosuppressed patients with VL warrants some consideration of the host when discussing the efficacy of AmBisome as treatment of

this disease. These data strongly suggest that the immunosuppressed host with VL is not able to achieve the same response with AmBisome therapy in long-term follow-up. It is noteworthy that 81% of the immunosuppressed patients were HIV-positive. Those patients coinfecting with HIV may represent a special subgroup, and generalization of their responses to therapy for all immunosuppressed patients may not be appropriate. The suggestion has been made that multiple courses of treatment and/or long-term maintenance therapy may be needed to prevent relapse in the immunocompromised patient [21]. The NDA submission did not provide data to support or refute this possibility.

### General Issues in Design of Clinical Trials

Controlled, prospective clinical trials using FDA-approved comparators are the standard for the evaluation of drugs to treat infections. For certain circumstances including some tropical and/or parasitic infections, this type of study may not be possible because of the lack of approved agents. The analysis of the data supporting the approval of AmBisome for the treatment of VL used a historical control that was based on what is known about the efficacy of unapproved comparators such as the pentavalent antimonials. A historical control was acceptable in this indication because it was possible to determine that there was a consensus regarding the acceptable efficacy of an agent to treat VL and it was possible to quantify and verify parasitological identifications and end points. Despite the lack of an active concurrent control arm, these studies were larger than most reported series evaluating drug therapy for VL and provided an adequate follow-up interval that would capture most relapses. The availability of case reports for all patients in the pivotal studies permitted verification of evaluability and efficacy data as reported by the applicant. It also permitted a more stringent analysis of the data that supported the findings of the applicant.

For the purpose of FDA approval, it is also possible to conduct active-controlled studies with unapproved comparators. Such a comparator may be used if it represents the standard of care in the geographic region where the study is being conducted or as established by the peer-reviewed medical literature. It is highly desirable that the choice of comparator be agreed upon in advance, as should the intent to demonstrate whether the investigational agent is equivalent or superior to the comparator. The submission of such clinical trial results should be accompanied by literature that supports the choice of an unapproved comparator.

Data from trials conducted outside the United States may be used to support approval by the FDA. The study population should be applicable to the intended patient population in the United States. The requirements of the Declaration of Helsinki must be observed, and informed consent must be obtained and documented. Clinical trial design, conduct, and results are

reviewed for scientific integrity and verifiability, regardless of the location of the study.

The submission of published studies to provide supporting data to a report is one means of providing quantitative data for analysis by the medical reviewer. Prior submission of the planned protocol during the Investigational New Drug phase is strongly advised if this approach is elected. Early discussion of clinical trial design with the FDA allows the drug sponsor and the medical reviewer to appreciate the goals of a study and helps to meet the scientific and regulatory requirements of a subsequent NDA.

Many of the drugs developed to treat parasitic infections may be designated orphan drugs because they are for treatment of a rare disease. The orphan designation is based on an applicable patent in the United States only. Orphan designation and approval for marketing are two separate processes; the drug sponsor must apply for each separately. After approval of a drug that has been granted an orphan designation, the FDA will not approve another sponsor's marketing application for the same drug for the same indication for 7 years [22].

#### Specific Issues in the Study of VL

The differences in response to therapy, and, perhaps drug susceptibility, in different geographic areas make it difficult to extrapolate efficacy data from one region and/or species to another. Clinical trials of antileishmanial drugs should specify the region in which the patients are to be studied and make some attempt to identify isolates from the patients being evaluated to the species level. Although the present status of in vitro susceptibility testing remains investigational, the development of axenically cultured amastigotes for drug susceptibility testing holds promise for future clinical application [23, 24].

It is possible to include patients who have been previously treated. However, it is necessary to document the date(s) of prior courses of treatment to exclude patients without clearance of previously administered drugs.

Parasitological identification should be established by visualization of amastigotes and/or culture of appropriate tissue specimens. Patients should be followed up for an adequate period to detect relapses. Demonstration of parasite clearance at the end of therapy is particularly supportive. Clinical follow-up should extend to at least 6 months after the completion of treatment. Although there is some controversy regarding the importance of a positive tissue smear for the treated patient who is clinically well, there are also data that suggest that the risk of relapse in treated patients for whom amastigotes are visualized in repeated tissue samples is higher than that in those for whom amastigotes are not visualized. The most conservative way to resolve this issue is to define overall success as clearance of parasites at the end of therapy and no relapses at long-term follow-up.

The immunosuppressed host with VL is a special case. Although there exists speculation regarding the role of repeated

courses of therapy or of maintenance therapy, clinical studies demonstrating efficacy of such regimens are needed.

#### Conclusion

AmBisome is the first drug approved in the United States for the treatment of VL. Discussion of the nature of the application submitted to support this approval highlights certain important aspects of clinical trial design that are pertinent to the development of drugs for the treatment of tropical diseases. There exists a wide range of possibilities in the design of clinical trials to evaluate drugs for the treatment of these often rare infections. The Division of Special Pathogens and Immunologic Drug Products, FDA, encourages early dialogue in the development of these products to best meet the regulatory requirements to demonstrate drug safety and efficacy.

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