8.2 Indication # 2 : PROPHYLAXIS against systemic fungal infections.

For this indication the applicant submitted data from 2 double blind, controlled studies. One was performed in bone-marrow transplant recipients and one in liver transplant patients.

8.2.1 Trial #104-13: AmBisome versus Placebo for the Prophylaxis of Fungal Infections.

8.2.1.1 Protocol

8.2.1.1.1 Objective/Rationale To evaluate efficacy and safety of AmBisome for the prevention of fungal infections in patients who will become predictably neutropenic due to therapy for their underlying cancer or bone marrow transplant allograft.

8.2.1.1.2 Design A prospective, randomized, double blind, placebo-controlled trial. This study was conducted in England and Ireland from 1992 through 1994. It was not conducted under a US IND.

8.2.1.1.3 Population Patients receiving chemotherapy for the treatment of acute lymphoblastic or myeloid leukemia, OR patients undergoing bone marrow transplantation for hematological malignancies. Only those patents receiving a dose of chemotherapy that is known to consistently cause neutropenia were to be included. Patients with a diagnosis of graft-versus-host disease and receiving prednisolone therapy in excess of 100 mg/day were eligible.

Patients were excluded if they had: 1.) clinical or other evidence that indicated a deep or disseminated fungal infection prior to enrollment; 2.) any patients who received systemic antifungal therapy within the last 28 days prior to study enrollment; 3.) neutrophil count > 0.5 x 10^9/L for at least 3 successive days.

8.2.1.1.4 Procedures Patients were randomized to receive either AmBisome 2 mg/kg or a placebo composed of glucose 50 mg/L, Vamin 9 glucose,
The vitamins and intralipid made the placebo visually similar to AmBisome.

Dosing: The study drug was administered over 1 hour daily. AmBisome was administered at a dose of 2 mg/kg three times per week (Monday, Wednesday, Friday).
Duration: Dosing of the study preparations was to commence on day one of the chemotherapy regimen and to continue until an end-points was reached.

Randomization was performed separately for each site.

8.2.1.1.5 Endpoints:

1) Recovery of the neutrophil count to greater than 0.5 x 10^9/L for at least three successive days; OR
2) A serious and non-resolving adverse event occurs, OR
3) A systemic fungal infection occurs (defined as a patient with a persistent fever of greater than 38°C for for which there is an unknown etiology and which has failed to respond to adequate systemic broad spectrum antibacterial and/or antiviral therapy); OR
4) The patient requests withdrawal; OR
5) The investigator decides that withdrawal is in the best interest of the patient; OR
6) For patients with GVHD, a daily steroid requirement of less than 100 mg of prednisolone.

Comment: *The above would be points during the conduct of the study when the study drug would be discontinued. The protocol stated that "the primary efficacy evaluation would be based upon the number of patients in each treatment arm who completed dosing without early withdrawal, following the first course of chemotherapy; and for patients undergoing multiple courses, the number of courses without a fungal infection will be compared". The protocol did not define fungal endpoints.*

8.2.1.1.6 Statistical considerations: These are not stated in the protocol beyond the number of patients to be enrolled (160 patients, total).

8.2.1.2 Results

APPEARS THIS WAY
ON ORIGINAL

8.2.1.2.1 Patient Disposition, Comparability

A total of 80 patients were randomized to AmBisome and 90 to placebo. Seven patients never received treatment, and were excluded from both safety and efficacy analyses. Five of these were in the AmBisome group. No information was available for one of the five, three had delay or cancellation of chemotherapy/transplantation, and one had received ketoconazole as prophylaxis. Two patients randomized to placebo were never treated; one lacks any data the other was re-randomized later on. Thus, 75 patients were evaluated on the AmBisome arm and 88 on the placebo arm.

8.2.1.2.2 Efficacy endpoint outcomes

APPEARS THIS WAY
ON ORIGINAL

8.2.1.2.2.1 Protocol Deviations

Protocol dosing started before or after chemotherapy was initiated. This was due to the procedure of initiating study drug on a Monday, Wednesday, or Friday schedule. Patients on AmBisome were started before in 25% of cases and after in 73% of cases. Similar rates were seen for the placebo group, 36% and 63%, respectively. Each group

had one patient who never received chemotherapy during the study period.

Patients enrolled with "proven" fungal infections included 5 in the placebo group (2 blood, 1 sputum, 2 pharynx grew C albicans) and 2 In the AmBisome group (2 stool cultures grew C. albicans).

Comment: *Only the two blood cultures would be considered indication of true systemic infection. The other cultures may only represent colonization.*

Prior systemic antifungal therapy was received by 4 patients, one in each group.

Two patients (01002 and 01004) listed as AmBisome patients received placebo. These patients were analyzed as placebo patients. It is suspected that the assignment of two patients at site 02 were known to the person completing the CRF. The applicant is unable to determine whether this person was directly involved with the administration of study drug and treatment of the patient. Furthermore, the CRF indicates that these two patients received 500 mL of saline, not placebo as defined above.

8.2.1.2.2.2 Reasons for Treatment Discontinuation

Table 8.2.1 Reasons for Treatment Discontinuation

	AmBisome N = 75	Placebo N=88
Neutrophils Recovered	27 (36%)	30 (34%)
Suspected Fungal Infection	25 (33%)	36 (41%)
Severe Adverse Event/Death	6 (8%)	3 (3%)
Intercurrent Illness (Venocclusive disease & renal failure)	1 (1%)	0 (0%)
Investigator Decision/Patient Request/Other*	16 (21%)	19 (22%)
Localized Fungal Infection (Suspected or confirmed)	1	9
Voluntary withdrew Reaction to first dose Toxicity	0 1 0	4 0 1
Venocclusive Disease	1	0
Neutrophils Recovering	1	2
Allergic Reaction	0	1
Miscellaneous	3	2
* These three reasons have been combined for this summary table. The subcategories refer to sponsor interpretation of investigator comments on the CRFs.		

Comment: *The above reasons were those listed on the CRF by the investigator. Information on severe adverse events and death are provided below. Review of line listings agrees with the above.*

APPEARS THIS WAY
ON ORIGINAL

8.2.1.2.2.1 Patient Characteristics

The majority of patients had a primary diagnosis of either acute or chronic myeloid leukemia (66% placebo; 56% AmBisome). Most patients received a bone marrow transplantation: 83% and 85% in the placebo and AmBisome groups, respectively. No major differences were seen between study groups at pre-treatment. For laboratory values at baseline, the groups were comparable, except for creatinine. Although the group means did not differ, more AmBisome-randomized patients had increased serum creatinine values than placebo patients at pre-treatment (p=0.01).

Demographics: The two groups were balanced for demographic characteristics, with the exception of gender. The median age of these subjects was 39.3 years with a range A majority of subjects were Caucasian (90.7%) and male (62.6%).

8.2.1.2.3 Efficacy Evaluation: Results

Table 8.2.2 shows the applicant's evaluation of fungal events, which includes "fungal colonizations".

TABLE 8.2.2: Number of Patients with Fungal Infections and Colonizations

	Placebo (N = 88)	AmBisome (N = 75)	P value
All fungal isolates	30 (34.1%)	11 (14.7%)	0.01
Proven fungal infections	4 (4.5%)	1 (1.3%)	NS
Colonizations	18 (20.5%)	10 (13.3%)	0.29
Urinary Infection	7 (8.0%)	0	0.013
Stool Infection	8 (9.1%)	6 (8.0%)	NS
Concomitant Systemic Antifungal Treatment	21 (23.9%)	21 (28.0%)	NS
Fever of Unknown Origin	11 (12.5%)	3 (4.0%)	NS

Comment: *The protocol did not specify what the primary endpoint would be, other than "fungal infection". Prevention of proven fungal infections and subsequent improvement in mortality is the most desirable outcome for assessing the efficacy of an antifungal for prophylaxis. The number of deaths was similar in both groups, placebo (12), AmBisome (11). The number of proven infections was low (a total of 5); therefore, it is problematic to evaluate this endpoint as an outcome. The documented infections were described as follows: AmBisome: 1 positive culture for C. albicans on a bronchoscopy specimen; Placebo: 3 blood cultures from which Candida species were isolated and one peritoneal culture with Aspergillus fumigatus. The positive culture from the bronchoscopy specimen would not be considered strong evidence for serious, invasive Candida pneumonitis.*

A confounding factor in this study was that approximately one quarter of the patients were receiving concomitant, systemic antifungal therapy. This would tend to decrease the overall number of infections. Review of the other positive culture sites such as urinary colonization reveals numerically fewer positive cultures among patients receiving AmBisome. However, colonization does not necessarily lead to serious

systemic infection. While it may be tempting to utilize fever as a surrogate for fungal infection, it can only be suggestive of infections. In this case, a very low number of these events limits the ability to distinguish a meaningful difference.

In general, this study is supportive of the utility of AmBisome in the prevention of fungal infection, but does not prove the case.

8.2.1.3 Safety Comparisons

APPEARS THIS WAY
ON ORIGINAL

8.2.1.3.1 Extent of Exposure:

TABLE 8.2.3: Study Drug Duration:

Number of Doses	AmBisome N=75	Placebo N=88
Single Dose Only	6 (8.0%)	2 (2.2%)
2 - 3 doses	3 (4.0%)	9 (10.2%)
4 - 6 doses	10 (13.3%)	18 (20.5%)
7 - 9 doses	25 (33.3%)	31 (35.2%)
> 9 doses	31 (41.3%)	28 (31.8%)

Comment: *Overall, the exposure was similar. There was a higher number of patients receiving greater than nine doses of AmBisome than placebo. The average length of neutropenia was similar for each group, 4.0 vs 3.8 days for the AmBisome and placebo groups, respectively. It appears that this variable did not bias the duration of therapy.*

8.3.1.3.2 Adverse Events Overall:

APPEARS THIS WAY
ON ORIGINAL

Table 8.2.4: Overall Adverse Events:

Body System/ Adverse Event	AmBisome N=75	Placebo N= 88
Body As Whole		
Abdominal Pain	10 (13.3%)	9 (10.2%)
Allergic Reaction	4 (5.3%)	2 (2.3%)
Back Pain	3 (4.0%)	2 (2.3%)
Cell-Mediated Immunological Reaction	3 (4.0%)	5 (5.7%)
Chest Pain	3 (4.0%)	5 (5.7%)

Fever	31 (41.3%)	40 (45.5%)
Headache	6 (8.0%)	9 (10.2%)
Influenza-like Symptoms	4 (5.3%)	0 (0%)
Mucositis (NOS)	13 (17.3%)	25 (28.4%)
Pain	2 (2.7%)	8 (9.1%)
CARDIOVASCULAR		
Hypertension	6 (8.0%)	1 (1.1%)
DIGESTIVE		
Constipation	4 (5.3%)	8 (9.1%)
Diarrhea	23 (30.7%)	31 (35.2%)
Dyspepsia	4 (5.3%)	6 (6.8%)
Jaundice	6 (8.0%)	5 (5.7%)
Nausea	31 (41.3%)	39 (44.3%)
Stomatitis	4 (5.3%)	5 (5.7%)
Vomiting	21 (28.0%)	25 (28.4%)
METABOLIC & NUTRITIONAL		
Hypokalemia	3 (4.0%)	6 (6.8%)
NERVOUS		
Dizziness	4 (5.3%)	2 (2.3%)
RESPIRATORY		
Dyspnea	1 (1.3%)	3 (3.4%)
SKIN		
Enanthema	0 (0%)	4 (4.5%)
Folliculitis	3 (4.0%)	4 (4.5%)
Rash	13 (17.3%)	14 (15.9%)
Rash Erythematous	4 (5.3%)	5 (5.7%)
UROGENITAL		
Renal Function Abnormal	3 (4.0%)	2 (2.3%)

NOTE: above AES listed for more than 3 events.

Comment: *The adverse events listed above were reported regardless of relationship to study drug and include events which are characteristic of patients with underlying neutropenia. The events seen in at least 10% or more of the patients were fever, nausea, diarrhea, vomiting, mucositis, rash, abdominal pain, headache. These events occurred at similar rates for both groups.*

The sponsor presented the events according to whether they were judged to be related to study drug by the investigator. The following table displays these events reported in 5 or more patients.

Table 8.2.5: Most Frequent Adverse Events Judged to be Related to Study Medication

Adverse Event	AmBisome N= 75	Placebo N = 88
Fever	12 (16.0%)	13 (14.8%)
Diarrhea	8 (10.7%)	9 (10.2%)
Rash	8 (10.7%)	9 (10.2%)
Nausea	8 (10.7%)	7 (8.0%)
Abdominal Pain	5 (6.7%)	4 (4.5%)
Vomiting	4 (4.5%)	5 (5.4%)

8.2.1.3.3 Withdrawals Due to Adverse events/toxicities Of interest are the patients who only received one dose of study drug. Five patients receiving only one dose of AmBisome experienced a reaction during or after their first dose of study medication that resulted in discontinuation.

Patient 01031: allergic reaction to first dose
Patient 02025: following first dose, experienced wheeze, hypertension and flushing
Patient 03035: after first dose, experienced headache, palpitations and tremor. Investigator judged this as a reaction to the first dose of study medication.
Patient 04020: Within seconds after initiating infusion, patient experienced chest tightness, difficulty breathing and erythema. The investigator judged this as an allergic reaction to the study mediation and withdrew the patient from study. The patient recovered 2-3 hours later.
Patient 04029: This was documented by the investigator as a severe reaction to the first dose of study medication.

One of the patients receiving placebo was discontinued due to an allergic reaction.

Patient 01036: This patient experienced an allergic reaction and was withdrawn from the study.

Comment: *Only two of the above events had signs or symptoms related to the respiratory system.*

8.2.1.3.4 Serious Adverse Events: Ten recipients of AmBisome and 9 Placebo recipients experienced serious adverse events.

AmBisome Treatment: Four patients experienced an allergic or other reaction to the first dose of AmBisome (patients 01031, 02025, 04020. 04029). All four of these patients were discontinued from the study and recovered thereafter. Four other patients (patients 01032, 02005, 02012, and 03005) had an outcome of death following a serious adverse event. Patient 01031 had pyrexia during three weeks of AmBisome treatment at which time study drug was withdrawn. This patients was administered open-label amphotericin B but developed pneumonia eight days later and died of probable Aspergillus pneumonia 21 days after discontinuing AmBisome. The other three patients died of venocclusive disease, ARDS and cerebral hemorrhage, respectively, within 3 weeks of completing AmBisome therapy. No death appeared to have been related to AmBisome, as discussed in section 8.2.1.3.5.

Placebo Treatment: Six of the nine patients on placebo who experienced a serious adverse event had an outcome of death. Two of these patients had respiratory problems leading to death: patient 02007 experienced pleural effusion with positive culture for Candida krusei, and patients 02029 had respiratory failure secondary to pulmonary infiltration. Two other deaths (patients 03012 and 05007) were a result of sepsis and one death was due to liver failure. Patient 04005 experienced pulmonary hemorrhage and respiratory failure a day or two after stopping study medication. The patient was thrombocytopenic throughout the study period (two weeks in duration) and died three days after the last dose of study medication. These six deaths are presented in more detail in section 8.2.1.3.5. Two patients (03007 and 03015) had renal impairment; one recovered while on study drug while the other discontinued study medication with some further complications. Venocclusive liver disease was diagnosed in Patient # 03020 during the post-treatment period. This patient underwent a liver transplant several weeks later.

Comment: *Since underlying disease is significant in these patients serious events are expected. There was a similar number in each treatment group; however, allergic reaction seemed to be associated with AmBisome infusion.*

8.2.1.3.5 Deaths: Twenty-three (23) of the 163 patients enrolled in this study died,

placebo (12), AmBisome (11). Causes of deaths are outlined below.

Table 8.2.6: Causes of Deaths

	Placebo (N=88)	AmBisome (N=75)
Disease Progression	3	3
Disease Related Complications	8	5
Intercurrent/Unrelated Event	1	3
TOTAL	12 (13.6%)	11 (14.7%)

Comment: *A review of causes of death agrees with the applicant's evaluation. Many of these patients had serious underlying diseases. Two deaths were listed as possibly related to study drug therapy (one in each treatment group).*

Patient 02012 *(AmBisome) a 31 y.o. female, who underwent a bone marrow transplant, was diagnosed with S. epidermidis. She was treated with immunosuppressants, antiviral and antibiotic medications, medications for gastric protection, nutritional support, as well as, antiemetic and bronchodialating medications. During the trial the patient experienced the following severe events: pyrexia; progressive bilateral pulmonary infiltrate leading to respiratory failure; renal failure. AmBisome was discontinued and approximately 3 weeks later the patient died of acute respiratory distress syndrome of unknown etiology. Autopsy is pending.*

Patient 03032 *(Placebo), a 36 yo male, underwent a bone marrow transplant. Some mild adverse events occurred during the study including retro sternal pain, generalized body pain, abdominal pain and nausea. Ten days after the patient was discontinued from study drug, she died due to increased liver enzymes and renal failure. Ultra sonic investigation was suspicious for CGL infiltrations of both kidneys. The investigator felt the patients's death was possibly related to study drug.*

None of the deaths appeared to be directly related to either therapy.

8.2.1.3.6 Laboratory Abnormalities:
For the clinically significant laboratory toxicities, as defined previously, no difference was detected between groups for nephrotoxicity, hypokalemia, hypernatremia, hyponatremia, increased alkaline phosphatase, hyperbilirubinemia, increased AST, or anemia.

Comment: *Further analysis based on changes in creatinine from baseline, revealed overlapping curves. This placebo controlled trial did not demonstrate an increased*

incidence of nephrotoxicity due to AmBisome in this patient population.

8.2.1.4 Reviewer's Comments/Conclusions of Study Results

This placebo controlled trial was not designed with a pre-specified efficacy endpoint. As has been discussed in Section 8.3.1.2.3, proven or documented systemic fungal infections are of most interest. Colonization of the urinary tract or stool, fever of unknown origin and concomitant systemic antifungal treatments were listed as outcomes which might be related to the outcome of interest. There were no statistically significant differences for these events except for urinary tract infections (significant using an unadjusted p-value). The difference in proven infections favored AmBisome: 1 infection vs, 4 for the amphotercin B group. A confounding factor was that approximately one quarter of the study patients were receiving concomitant, systemic antifungal therapy during the study. This would tend to decrease the overall number of infections. This study is supportive of the activity of AmBisome for this study population; however, there were too few proven fungal infections (4%) to permit definitive evaluation of the efficacy of AmBisome for prohpylaxis of fungal infections.

The safety of AmBisome was similar to the control group, placebo. However, it should be remembered that these patients had serious underlying illnesses. While a small number of allergic reactions were seen with AmBisome, AmBisome did not appeared to be tolerated.

8.2.2 Trial #104-08: AmBisome versus Placebo for the Prophylaxis of Fungal Infections in Liver Transplant Recipients

8.2.2.1 Protocol

8.2.2.1.1 Objective/Rationale

To evaluate the efficacy and safety of AmBisome for the prevention of fungal infections in patients undergoing orthotopic liver transplantation.

8.3.2.1.2 Design

This was a multicenter, prospective, randomized, double-blind, placebo controlled study. It was conducted in Sweden and Finland between February 1991 and April 1992. It was not conducted under a US IND.

8.2.2.1.3 Population

Patients scheduled to undergo orthotopic liver transplantation were given the opportunity to enroll in this study. Patients of any age who were scheduled for OTL were included.

Patients were excluded if they had proven or probable invasive or disseminated fungal infections or known intolerance to amphotericin B.

Comment: *The original protocol was to include bone marrow transplant recipients as well as liver transplant recipients. This study reports only the experience in the liver transplant patients.*

8.2.2.1.4 Procedures

Patients were randomized based on a block randomization schedule (size 4) constructed for each study site. Patients received either AmBisome 1 mg/kg IV daily or placebo to be administered . Five days of prophylactic therapy was to be given following transplantation.

All patients were to undergo the following pre-study evaluations: history and physical examination, surveillance cultures (urine, throat, stool, bile and peritoneal fluid), and hematology and chemistry assessments. During the protocol, BUN, serum creatinine, sodium and potassium were to be obtained 3 times per week; other chemistry and hematology assessments were to be obtained once per week.

Fungal infection surveillance and diagnosis: Serological and culture tests were to be performed before initiation of the therapy and once weekly during treatment.

Comment: *Candida antigen and serology testing are not considered diagnostic of a*

systemic fungal infection. These tests are not approved in the US, and are not accepted by the MSG as the basis for proven infections.

All patients were to be treated for 5 days after liver transplantation. Repeat 5 day prophylactic therapy, for the first 3 months after liver transplantation, was permitted for each of the following conditions: retransplantation; major surgery; therapy resistant rejection or second rejection treatment; septicemia; CMB infection.

Comment: *The version of the protocol included in the study report only allows for initial prophylaxis and one month follow up. No additional information was provided on subsequent courses of prophylaxis as outlined above in the protocol. Additionally, no information on follow up at 3 months was given.*

Study drug therapy could be discontinued for any of the following reasons:

- Evidence of a proven deep or disseminated fungal infection;
- Evidence of a suspected deep or disseminated fungal infection after 72 hours of persistent fever unresponsive to antibiotic and or antiviral medication;
- Completion of 5 days of prophylaxis with study drug.
- Severe adverse events;
- Patient request;
- Investigator decision.

8.2.2.1.5 Endpoints

Prophylactic efficacy of the study drug was to be primarily determined by the investigator's evaluation of disseminated fungal infection occurring during the prophylactic treatment period.

Success: No evidence of suspected or proven deep or disseminated fungal infection.
Failure: Evidence of suspected or proven deep or disseminated fungal infection.

Criteria for failure:

Suspected fungal infection: 72 hours of fever unresponsive to antibiotic and/or antiviral medication with no clearly identifiable etiology.

Proven infection criteria:

- Positive blood culture
- Cultures from sterile body site (peritoneum, pleura, bile)
- Deep organ biopsy (kidney, liver, spleen, lung)
- Pulmonary x-ray finding plus bronchoscopy findings (culture, microscopy, biopsy) for Aspergillus species infections

Suspected infection criteria:

- Fever and colonization of 3 body sites by Candida species other than *Candida albicans*
- Serologic evidence
- Free Candida antigen titer ≥ 1:4
- Pulmonary x-ray finding coupled to bronchoscopy findings (culture, microscopy, biopsy) for Candida.

Comment: *Liver transplant surgery interrupts the integrity of the abdominal cavity. In addition the integrity of the bowel is interrupted at the site of anastomosis with the bile duct, many patients also have Roux-en-Y procedures, and most have some kind of bile duct stent or T-tube drainage. It is agreed that proven fungal infections are those where a culture was obtained from a sterile site; however, it is not clear that the peritoneum (in the case of secondary closure of the abdominal wound) or bile cultures obtained from T-tube drainage would represent proven fungal infections versus colonizations. These should be considered as "suspected" infections.*

Note, also that the applicant changed the final efficacy variable described in results below.

8.2.2.1.6 Statistical considerations

This study was originally designed to study both BMT and liver transplant recipients. Prophylaxis was to be considered successful if development of deep fungal infection did not occur. Sample size calculations were based on the ability to detect differences in the emergence of fungal infections between treatment arms for both populations. The sponsor concluded that 80 patients per arm were necessary to detect the predicted difference in treatment effect (90% power and p-value < 0.01). A preliminary analysis was to be performed when 40 BMT and 40 liver patients were evaluable.

Comment: *The application reports results only for the orthotopic liver transplant recipients. Bone marrow transplant recipients are not described in this report.*

8.2.2.2 Results

To assess the efficacy of AmBisome, the applicant re-defined efficacy as the development of fungal infection within 30 days post-transplant based on positive cultures from blood, sterile body sites or deep organ biopsy culture or histology. For aspergillosis, pneumonia on x-ray plus positive BAL culture or lung biopsy was considered diagnostic.

Comment: *This was a change from the protocol definition as described above. In addition, the results of this study were presented in the form of the journal article published in Transplantation: Tollemar J, etal. Liposomal Amphotericin B Prevents Invasive Fungal Infections in Liver Transplant Recipients. Transplantation. 59, 45-40. 1995. The applicant subsequently submitted a limited SAS data base as requested by the FDA. Because of the age of the study, the CRF requirement was waved.*

8.2.2.2.1 Patient Disposition, comparability

A total of 86 patients were randomized, 59 in Sweden and 27 in Finland. One patient died during surgery; thus, 85 (42 placebo and 43 AmBisome) patients received at least one dose of study drug and were evaluated for safety. One patient did not have adequate preoperative data to be evaluated for efficacy. Thus, 84 were potentially evaluable for efficacy. An additional 7 patients were withdrawn from the study before 5 days of therapy (4 placebo, 3 AmBisome) and were not considered evaluable for efficacy leaving 77 evaluable (37 placebo, 40 AmBisome). Three placebo patients were removed because of suspected fungal infections compared to one in the AmBisome group.

TABLE 8.2.7. Disposition of Study Patients

	TOTAL	Placebo	AmBisome
All randomized	86	43	43
Never received drug	1	1	0
Evaluable for safety	85	42	43
No baseline efficacy data	1	1	0
Efficacy Intent-to-treat	84	41	43
<5 days therapy	7	4	3
Evaluable for efficacy	77	37	40
Alive at 30 days	76	36 (86%)	40 (93%)

8.2.2.2.2 Efficacy endpoint outcomes

8.3.2.2.2.1 Protocol Deviations

No other concomitant systemic antifungal therapy was permitted during prophylaxis. Topical treatment with Nystatin 400,000 IU 4 times daily was allowed, given orally or by NG tube. Nystatin was given to 31/42 (74%) placebo patients and 33/43 (78%) AmBisome patients.

Comment: *Additional information regarding protocol violations was not available in the submission.*

8.2.2.2.2.2 Reasons for Treatment Discontinuation

A total of 7 patients were withdrawn from study drug during the 5 day treatment period.

TABLE 8.2.8. Reasons for Treatment Discontinuation

Group	Day	Reason for Withdrawal/ Subsequent Antifungal Therapy
PLACEBO	4	Suspected hepatotoxicity
PLACEBO	4	Suspected FI/AmBisome
PLACEBO	4	Suspected FI/AmBisome
PLACEBO	4	Suspected FI/Fluconazole
AMBISOME	1	suspected FI at surgery/
AMBISOME	2	Amphotericin B
AMBISOME	1	Poor condition/Fluconazole
		Possible nephrotoxicity

Comment: *Discontinuations due to suspected fungal infections in the placebo group were based upon antigenemia. For the AmBisome group, this was based on clinical suspicion.*

8.2.2.2.2.1 Patient Characteristics

The distribution of patient characteristics is similar between treatment groups except for gender. There were more women in the placebo group and more males in the AmBisome group.

Table 8.2.9: Patient Characteristics

	Placebo	AmBisome
Number of patients (Sweden/Finland)	37 (25/12)	40(28/12)
Gender (Female/male)	23/14	15/25
Age (median (range))	40	42
Donor gender (F/M)	23/17	22/15
Chronic active hepatitis	9	7
Primary biliary cirrhosis	8	8
Metabolic disease	8	7
Carcinoma	4	8
Primary sclerosing cholangitis	2	5
Acute liver failure	4	4
Retransplantation	2	1

8.2.2.2.3 Efficacy Evaluation

The results of the applicant's and FDA's evaluation of success is presented in Table 8.2.10.

TABLE 8.2.10. Proven Invasive Fungal Infections

	Placebo N=37	**AmBisome 1 mg/kg N= 40**
Applicant's result	**6 (16.2%)**	**0 (0%)**
FDA's result using applicant's criteria	**6 (16.2%)**	**1 (2.5%)**
FDA's result	**3 (8.1%)**	**0 (0%)**

Comment: *The applicant supplied the FDA with additional SAS data sets for the primary outcome variables. In addition, a list was prepared by the sponsor which presented a narrative of the infections. There were 4 infections, referred to as peritonitis in the journal article. However, these diagnoses appeared to be based on positive bile cultures taken from T-tubes. These "infections" may have been colonizations rather than invasive infections. If one were to include all positive bile cultures in the "proven" category, an additional infection would be added to the AmBisome column. This change would result in a statistically insignificant result,*

although the numeric trend would favor AmBisome.

Non-Prophylactic Systemic Antifungal Therapy:
Among placebo patients, 13 of 41 in the intent-to-treat group received additional, non-prophylactic systemic antifungal therapy. Six received such therapy for treatment of proven infection, three were treated for suspected fungal infections after discontinuation of study drug prophylaxis and the reaming four for suspected fungal infections in the post-prophylaxis period. Among AmBisome patients, 6 of 43 in the intent-to-treat received additional, non-prophylactic systemic antifungal therapy (p=0.069). Two were prematurely discontinued, one because of clinical evidence of fungal infection suspected at operation and one because of poor clinical status. The remaining four patients were treated for suspected fungal infection in the post prophylactic period.

Comment: *Slightly more patients in the placebo group were treated with additional systemic antifungal therapy.*

While this data is supportive of AmBisome's antifungal activity, the difference in the number of documented infections was too small to evaluate AmBisome's efficacy for prophylaxis of fungal infections.

8.2.2.3 Safety Comparisons

8.2.2.3.1 Extent of Exposure Five days of prophylactic therapy was received by all but 7 patients in the study.

Comment: *The distribution of early withdrawals was similar in each arm.*

8.2.2.3.2 Adverse Events Overall and Withdrawals Due to Adverse Events
Three patients in the AmBisome group experienced adverse events related to the drug. One patient had transient thrombocytopenia, another suffered from suspected nephrotoxicity and also suffered from transient thrombocytopenia, and a third patient experienced back pain related to the AmBisome infusion. When the infusion rate was lengthened in the latter patient, back pain disappeared and the patient received a full course prophylaxis.

One patient in the placebo group experienced hepatotoxicity.

Comment: *This information was taken from the journal article. No supportive line listings were provided by the sponsor, thus further comment cannot be made.*

8.2.2.3.3 Serious Adverse Events

Comment: *Line listings, other than those documented above, were not provided by the applicant.*

8.2.2.3.4 Deaths

There were 6 deaths among the 42 patients who received placebo (86% survival rate). Among the 43 AmBisome patients, there were three deaths for a 93% survival rate. No deaths were attributable to study drug or fungal infection.

TABLE 8.2.11. Causes of Death

Group	Patient ID	Cause of Death
PLACEBO	218	Rejection Reaction
PLACEBO	232	Intercurrent/Unrelated Illness
PLACEBO	210	E.coli Septic Shock
PLACEBO	209	Necrotizing Pancreatitis
PLACEBO	204	Intercurrent Illness
PLACEBO	201	Cerebral Hemorrhage
AMBISOME	237	Intercurrent/Unrelated Illness
AMBISOME	212	Disease Related Complications
AMBISOME	202	Multi-Organ Failure

Vol 4.1, sect 11.2.3

Comment: *FDA review of death narratives provided by the applicant concurs with the data summarized in the table above. Based on the information supplied, none of the deaths were attributable to study drug or fungal infection.*

8.2.2.3.5 Laboratory Abnormalities

The only significant difference between the placebo and AmBisome groups was the mean serum alkaline phosphatase values at 30 days post-transplant. AmBisome treated patients had 3.3 times the upper limit of normal compared with 1.5 times for placebo group patients ($p<0.01$). In a subpopulation of 20 Finnish patients, the proportion of skeletal and liver isoenzymes of alkaline phosphatase were similar for the two study drug groups. No other laboratory tests differed significantly between groups. AmBisome patients had slightly higher mean serum creatinine levels at baseline and the difference remained consistent throughout the 30-day period with no evidence of nephrotoxicity.

8.2.2.4 Reviewer's Comments/Conclusions of Study Results

This placebo controlled endpoint evaluation was performed without a clearly defined pre-specified endpoint. Review of proven systemic infections revealed uncertainties regarding the assignment of 3 patients in the amphotericin B group. Specifically, these

cases were diagnosed based on positive cultures (Candida species) from T-tube drainage. These could represent colonization rather than true systemic infection. No additional information upon which to judge the potential systemic nature of these infections was presented. Based on our reassessment, the number of proven infections was zero for the AmBisome group and 3 for the amphotericin B group. The numbers are too small to support an efficacy claim for prophylaxis in this patient population. A definitive estimate of the prevention of proven, systemic fungal infections cannot be made based upon the data presented.

No safety concerns were raised by this study, except the unexplained elevation in alkaline phosphatase. No clinically significant effects were observed related to this finding.

8.3 Indication # 3: Treatment of systemic and/or deep mycoses including *Aspergillus* species and *Candida* species

For this indication the sponsor submitted controlled and uncontrolled study data. The sponsor proposed two treatment indications for infections with Aspergillus or Candida species, one for initial treatment and one for "second-line" therapy (in patients refractory or intolerant to amphotericin B); both can be addressed by the same studies.

8.3.1 Trial # 1 104-00: An open-label, uncontrolled compassionate use trial of AmBisome

8.3.1.1 Protocol

8.3.1.1.1 Objective

To evaluate the safety and efficacy of AmBisome in treating patients with invasive fungal infections for whom there is no alternative.

8.3.1.1.2 Design

The study design was multicenter, open label and uncontrolled.

Comment:
This was a compassionate use program.

8.3.1.1.3 Population

Patients with severe and life-threatening invasive fungal infections were eligible for treatment with AmBisome if they had evidence of an invasive fungal infection for which conventional amphotericin B therapy was not feasible for reasons of toxicity, previous inadequate response, and/or renal insufficiency.

Comment:
The protocol did not contain specific criteria for what constituted treatment failure or intolerance.

8.3.1.1.4 Procedures

Patients were to receive AmBisome infused over 30 minutes to one hour. Investigators were to determine AmBisome dose based on the type of fungal infection being treated and the patient's previous antifungal treatment. The protocol permitted dose escalation in increments of 0.2 mg/kg day up to a dose of 3 mg/kg.

The protocol required daily monitoring of vital signs, BUN, and serum creatinine. Other laboratory measurements (chemistry and hematology panels) were to be obtained prior

to therapy, weekly and within 24 hours of the last dose.

8.3.1.1.5 Endpoints

Efficacy was to be determined by the investigator's assessment of the resolution of clinical signs and symptoms of fungal disease in conjunction with the mycological culture results. An outcome of cure, improvement, or failure was to be determined based on a 48 hour post-therapy evaluation compared to pre-treatment findings.

For safety endpoints, the following selected adverse events were defined as follows:

> Nephrotoxicity: an increase of more than 100% of the baseline serum creatinine values.
>
> Hypokalemia: defined as serum potassium ≤ 2.5 mmol/L

8.3.1.1.6 Statistical considerations

To be considered evaluable for clinical outcome patients were required to have a clinical diagnosis of systemic fungal infection before drug treatment. The original protocol also required that patients receive at least seven days of therapy to be considered evaluable. The protocol was later amended such that patients who received more than one dose of AmBisome would be considered evaluable for efficacy.

For mycological evaluability, a fungal pathogen was to be identified by culture, serology, or microscopic examination of biopsy specimens.

Comment:
Except for cryptococosis and perhaps histoplasmosis, the use of fungal serologies are not reliable for diagnosing fungal infections.

Since this data was obtained from an uncontrolled compassionate use protocol, statistical methods to evaluate the study data were descriptive.

8.3.1.2 Results

To assess the efficacy of AmBisome, the applicant retrospectively categorized fungal infections as definitive or presumptive based on pretreatment clinical and mycological data. A definitive diagnosis of fungal infection required either a positive pretreatment fungal culture from a site considered normally sterile or a biopsy showing invasive fungal elements. For episodes with radiographic evidence of pulmonary aspergillosis, a positive bronchoalveolar lavage (BAL) or sputum culture for *Aspergillus species* was considered definitive. The sponsor's definition of a presumptive diagnosis included signs and symptoms of fungal infection with a positive culture from a nonsterile site such as stool or urine or a positive Candida culture from a BAL. Serologies were also

considered to be supportive of a presumptive infection.

Patients who did not qualify for either the definitive or presumptive category were categorized as "undefined."

Comment:
The protocol did not specify what constituted a definitive versus a presumptive diagnosis. This was decided retrospectively by the sponsor.

The applicant's definition of a definitive disagnosis is not in complete agreement with guidelines formulated by the Mycoses Study Group (MSG). For example, according to MSG criteria a definitive diagnosis of pulmonary aspergillosis requires positive histology and a positive culture or a negative histology with a positive culture obtained by invasive techniques. According to MSG criteria, a probable diagnosis of pulmonary aspergillosis includes two sputum cultures or one positive bronchoscopy specimen in an immunocompromised host with symptoms. Therefore the applicant's definition of definitive included a definitive or probable diagnosis by MSG criteria.

8.3.1.2.1 Patient Disposition, Comparability

A total of 133 patients were enrolled for the treatment of 140 separate fungal infections. One patient had two sites of fungal infection. Five patients were re-enrolled. One patient was treated for four episodes of fungal infection.

Comment:
Evaluating subsequent fungal episodes in re-enrolled patients is often difficult. Some patients who re-enrolled had the same type of fungal infection a second time, e.g., a patient with pulmonary aspergillosis during the first enrollment re-enrolled with pulmonary symptoms during a subsequent episode of neutropenia. In this case it was difficult to discern whether the first episode was completely eradicated or whether the patient was re-infected during a subsequent episode of immunosuppression.

Some patients did not have diagnostic procedures establishing infection during subsequent episodes. In some cases fungal cultures obtained prior to the first fungal episodes (perhaps months before the fungal episode of interest) were used to indicate the presence of infection. In FDA analyses these cases were considered nonevaluable.

8.3.1.2.2 Efficacy endpoint outcomes

Efficacy analyses were conducted for both mycological and clinical outcome as determined by investigators. The applicant also conducted an evaluation in which only infections in the definitive category were included.

Reasons for discontinuation of AmBisome, according to the investigator's assessment,

are listed in Table 8.3.1. The most frequent reasons for treatment discontinuation were resolution of infection or death. Based on investigator comments in case report forms, the applicant further subdivided the drug discontinuation category designated "other" (see Table 8.3.1).

Table 8.3.1. Reasons for discontinuation

REASONS FOR WITHDRAWAL	n 140 (Total)	(%) (100)
Infection resolved	47	(34)
Death	43	(31)
Adverse Event	2	(1)
Intercurrent Illness	4	(3)
Insufficient Clinical Improvement	4	(3)
Missing	3	(2)
Other	35	(25)
treatment success	7	
clinical improvement	11	
treatment failure	4	
toxicity	0	
drug not available	6	
disease progression	2	
patient withdrawal	3	
investigator decision	2	

Source: 14.1 page 36

Demographic and Baseline Characteristics

The majority of the 133 patients treated with AmBisome were male (62%) and white (89%). Median age was 37 Table 8.3.2 lists the reasons for enrollment. Approximately one-third had experienced amphotericin B nephrotoxicity and one-third amphotericin B treatment failure. The other third either had no reason recorded or had renal insufficiency that precluded the use of a nephrotoxic drug. The mean/median pretreatment serum creatinine was 155 and 104 µmol/L, respectively,

Table 8.3.2 Reasons for Enrollment in AmBisome Compassionate Use Program

Reason	Number (%) of Episodes
Amphotericin B failure	42 (30)
Amphotericin B nephrotoxicity	49 (35)
Renal Insufficiency	35 (25)
Other/Missing	14 (10)
Total	140 (100)

Source: Vol 14.1 page 38.

Efficacy

According to the sponsor's retrospective assessment, there were 92 episodes of definitive fungal infections.

Comment:

Many of the pulmonary infections with Aspergillus species were diagnosed using cultures obtained during bronchoscopy. Without lung biopsy or histologic confirmation, these diagnoses would be categorized as probable but not definitive according to Mycosis Study Group (MSG) criteria. In addition, some pulmonary aspergillosis infections were diagnosed based on one sputum culture. For a probable (MSG criteria) diagnosis, two sputum cultures are required; one sputum culture would support a possible diagnosis. Therefore some infections categorized as definitive by the sponsor would be possible according to MSG criteria.

The sponsor chose not to assess fungal infections that were considered to be presumptive or undefined by the investigator. Undefined infections had no recorded data that would substantiate a fungal diagnosis. In this program, presumptive diagnoses were based on fungal serologies of fungal cultures from nonsterile sites.

Comment:

For assessing treatment outcome it was reasonable to exclude the presumptive and undefined cases since initial diagnosis could not be reasonably substantiated.

Tables 8.3.3 shows the clinical response to treatment as assessed by the investigators. Outcome is reported by fungal infection type. The most frequent fungal infections were caused by *Aspergillus species*, *Candida species*, and *Cryptococcus neoformans*. The "other" category included fungi such as *Fusarium*, *Mucormycosis*, *Trichosporon beiglii* and others. Of the fungi comprising the other category, there were no more than one case for each fungal type (except for Madurella which had 2); most episodes in this category were nonevaluable. In effect, there are only data for infections with

Aspergillus, Candida and Cryptococcus species.

Table 8.3.3 shows the number of clinical responses as reassessed by FDA review of the data submitted. The percentage of successful responses by FDA analyses is somewhat less than that reported in the NDA.

Differences in the FDA's assessment of the endpoint as compared to the applicant's assessment are listed in Appendix A1-3.

Comment:
There were only 8 evaluable infections in the "other fungi" category which included a mixture of very different types of infection; therefore, I did not reassess clinical or mycologic outcome for this group when analyzing the efficacy data as a whole.

The line listings included microbiologic culture data, but listings of clinical and radiographic data were not included. This made it difficult to independently assess the clinical response to treatment. One must rely on the investigators' end of treatment evaluation.

Table 8.3.3. Clinical Outcome: Number (% of evaluable patients)

OUTCOME	ASPERGILLUS	CANDIDA	CRYPTOCCOCCUS	OTHER	TOTAL
Investigator					
Cured	12 (38%)	18 (72%)	6 (86%)	3 (38%)	39 (54%)
Improved	8 (25%)	3 (12%)	1 (14%)	5 (62%)	17 (24%)
Failed	12 (38%)	4 (16%)	0	0	16 (22%)
Total Evaluable	32	25	7	8	72
Not evaluable	6	10	2	2	20
FDA					
Cured	10 (32%)	17 (59%)	5 (63%)	-	-
Improved	5 (16%)	2 (7%)	0	-	-
Failed	16 (52%)	10 (34%)	3 (37%)	-	-
Total evaluable	31	29	8	-	-
Not evaluable	3	3	2	-	-

Source Vol 14.1 Page 87 Table 14.2.6

Mycological Outcome

According to the applicant's assessment, thirty-nine of the 92 "definitive" fungal infections had inadequate follow-up and were excluded from further evaluation for mycological outcome, leaving 53 episodes of definitive fungal infections with mycological follow-up. Of these 53 episodes of "definitive" fungal infections, the applicant concluded that 34 (64%) were mycologically eradicated. The applicant's and FDA's evaluation of mycological response for the three most frequent fungal infections occurring in this compassionate use program are shown in Table 8.3.4 below.

Table 8.3.4 Mycological Eradication

Outcome	Aspergillus	Candida	Cryptoccoccus	Other	Total
Applicant					
Eradicated	10 (43%)	17 (81%)	5 (83%)	2 (67%)	34 (64%)
Persisted	13 (57%)	4 (19%)	1 (17%)	1 (33%)	19 (36%)
Total	23	21	6	3	53
Not evaluable	15	14	3	7	39
FDA					
Eradicated	6 (27%)	9 (64%)	5 (83%)	ND	ND
Persisted	16 (73%)	6 (43%)	1(17%)	ND	ND
Total	22	14	6	ND	ND
Not evaluable	10	16	4	ND	ND

Source Vol 14.1 page 83 Table 14.2.3
ND = not done

8.3.1.2.3 Safety Comparisons

Safety data for this study includes 133 patients treated for 140 episodes of fungal infection. According to the sponsor the average duration of study treatment for 140 fungal episodes was 24.5 days. The mean cumulative dose received (based on 112 episodes) was 2620 mg.

Adverse Events

According to the applicant, in 91 of the 140 treated episodes, patients experienced at least one adverse event. In 51 of the 140 treated episodes, patients experienced at least one serious adverse event. The most frequent adverse events, occurring in at least 2% of the treated episodes and of any relationship or severity are shown in Table 8.3.5.

Table 8.3.5 Adverse Events Occurring in at least 2% of the treated fungal infections

CoStart Body System	Adverse Event	N total=140	%
Body as a Whole	Abdominal Pain	3	2.1
	Back Pain	3	2.1
	Cell-mediated immunologic reaction	7	5.0
	CMV infection	3	2.1
	Fever	3	2.1
	Fungal infection	12	8.6
	Sepsis	6	4.3
Cardiovascular	Cardiac arrest	3	2.1
Digestive	Nausea	6	4.3
	Vomiting	6	4.3
Hemic and Lymphatic	Acute Leukemia	4	2.9
Metabolic and Nutritional	Bilirubinemia	3	2.1
	Hypokalemia	6	4.3
	Creatinine increased	11	7.9
Nervous	Convulsions	3	2.1
Respiratory	Pneumonia	5	3.6
	Respiratory Insufficiency	8	5.7
Urogenital	Acute Renal Failure	5	3.6

Source: Vol. 14.1 Page 93 Table 14.3.3

Dyspnea and bronchospasm have been reported as infusion toxicities associated with the infusion of amphotericin B and liposomal amphotericin B products. There have been some concerns that the frequency of respiratory symptoms may be greater for the liposomal products, perhaps related to the presence of the liposomal component. Reports of dyspnea and bronchospasm may also represent possible allergic or anaphylactoid reactions to amphotericin B. Because of these concerns, case report forms for adverse events relating to respiratory insufficiency or other pulmonary-related events were reviewed.

There were 8 adverse events coded as respiratory insufficiency. In addition there were respiratory adverse events coded under the following terms: pulmonary edema (01010, 23001), massive alveolitis (09003), pulmonary embolism (27001), interstitial pneumonia (27002), and 5 cases of pneumonia. Brief narratives for these adverse events are listed below. From a review of case report forms it is apparent that most of these respiratory adverse events were more likely related to underlying disease and infections rather than an adverse effect of AmBisome infusion. None of these cases appeared to

have characteristics similar to those reported in the literature.

Comment:
It should be noted that the data listings were sometimes based on information reconstructed from medical notes and summaries. Copies of medical discharge summaries were sometimes included in the medical notes.

Cases of Respiratory Insufficiency

01008 No CRF (case report form) submitted, listed as **worsening** respiratory failure; investigator considered event not related.

06001 Presumptive pulmonary aspergillosis at baseline in a patient undergoing BMT (bone marrow transplantation) for AML (acute myelogenous leukemia); autopsy showed massive lung infarction. Investigator considered event not related

07002 Occurred in a patient undergoing BMT for AML, respiratory failure was due to pneumocystis pneumonia.

07004 Occurred in a patient undergoing BMT for CML. Respiratory decompensation requiring intubation occurred prior to treatment with AmBisome.

08002 Pulmonary and hepatic failure in a patient undergoing second OLT (orthotopic liver transplant) for sclerosing cholangitis. The patient was intubated pretreatment due to severe pulmonary aspergillosis diagnosed by BAL.

09010 Respiratory insufficiency in a patient undergoing BMT for multiple myeloma. Patient died due to respiratory failure from Aspergillus, present at baseline. This was a treatment failure. This patient had a previous life-threatening reaction to amphotericin B (fever and dyspnea) but no severe reaction to AmBisome.

09012 Progression of pulmonary aspergillosis

23001 Lung edema. This patient had pulmonary edema and fungal endocarditis at baseline.

25001 Patient with AIDS, suspected pulmonary candidiasis (BAL) with interstitial infiltrates pretreatment. Patient died of progressive respiratory failure after two doses.

Events coded under other CoStart terminology relating to respiratory compromise

01010 Pulmonary edema with massive hemoptysis. This patient had fluid overload and renal and hepatic failure at baseline. This patient had received only one dose of AmBisome. Investigator considered event to be unrelated to treatment.

09003 Massive alveolitis. Patient with ANLL (acute non-lymphocytic leukemia). This patient had a positive *C. tropicalis* blood culture and alveolitis at baseline. Autopsy showed disseminated *C. tropicalis*.

27001 Pulmonary embolism considered to be unrelated to drug. CRF not submitted.

27002 Interstitial pneumonia. This patient had CLL (chronic lymphocytic leukemia) and periorbital cellulitis due to Aspergillus species. Autopsy showed lymphocytic infiltrates in lungs.

In 3-month animal (rats) toxicity studies there was liver dysfunction associated with AmBisome. In order to determine whether liver toxicity was occurring in clinical trials, we reviewed CRFs of patients with adverse event listings consistent with symptoms and signs of liver toxicity. Narratives of these cases are listed below.

Cases with hepatic insufficiency

01010 Hepatorenal syndrome present at baseline

04002 Bilirubinemia, cholestasis, increased alkaline phosphatase levels considered possibly related. CRF not submitted.

08002 Hepatic failure, in a patient who had two liver transplants. Event considered not related to drug.

09012 Hyperbilirubinemia considered not related but AmBisome was dosed reduced because of this. Alkaline phosphatase and transaminases were not elevated. Received 13 days or 2 grams total of AmBisome.

31001 Hyperbilirubinemia, considered possibly related in a patient with AML and hepatosplenic candidiasis. Transaminases and bilirubin were elevated at baseline; lab abnormalities worsened near end of treatment. Assessed as related to disease, TPN, or possibly AmBisome

45001 Increased liver function tests (mild elevations of SGPT), considered possibly related.

Several patients receiving AmBisome experienced liver dysfunction or liver function test abnormalities; however, it was not possible to distinguish adverse drug effect from underlying disease in these patients with multiple serious medical conditions.

Serious Adverse Events

Fifty-one patients experienced at least one serious adverse event. Two patients had serious adverse events that were assessed as possibly drug related. Patient #13003 had polyneuropathy which was possibly related to underlying sepsis or drug, and patient #38001 had hypokalemia, thrombocytopenia and anemia which was considered possibly drug related. Other serious adverse events, that investigators considered to be unrelated to AmBisome, but were unusual or involved respiratory or liver dysfunction were reviewed. Narratives for these events are listed below:

01004 Hypotension and hypoxemia. This patient had pulmonary aspergillosis and Hodgkin's disease requiring intubation at enrollment secondary to ARDS. The patient died one hour after the first infusion of AmBisome due to hemodynamic failure. At autopsy the patient had pulmonary aspergillosis and a vegetation on the tricuspid valve. With the severity of the underlying disease it is difficult to discern a relationship to AmBisome.

03004 Pancreatitis in a patient who underwent a liver transplant. This patient died with acute pancreatitis

08002 Hepatic failure in a patient with a liver transplant secondary to sclerosing cholangitis.

24002 Cardiac arrest in a patient who underwent BMT for myelodysplastic syndrome. The patient had FUO and died during an exploratory laparotomy for abdominal pain and distention.

44001 Congestive heart failure in the setting of Aspergillus species endocarditis on a prosthetic valve.

In summary, one of these serious adverse events involved cardiovascular collapse after the first AmBisome infusion. It is difficult to determine if this was related to drug; the investigator considered the fatal adverse event to be unrelated to AmBisome. The patient was severely ill with ARDS requiring mechanical ventilation in an ICU. Pulmonary aspergillosis and tricuspid valve vegetations were discovered at autopsy.

Adverse Events Resulting in Drug Discontinuation

According to the applicant there were two patients who discontinued drug due to adverse events. Patient #21001, who had been vomiting at the time of enrollment, discontinued drug after 4 days due to severe vomiting. Patient #38001 developed severe anemia, thrombocytopenia and hypokalemia after 8 days of treatment with AmBisome. These events were considered possibly related to study drug.

Deaths

Fifty-three of the 133 patients died. The relationships of the deaths to AmBisome administration was considered not related or doubtful in all cases. Forty-six deaths occurred within 7 days of discontinuation of study drug.

8.3.1.3 Study 104-00: Overall Conclusions

An uncontrolled compassionate use study may be useful in providing safety data and in providing some assurance that a treatment is active, particularly if death is the usual outcome of no treatment. However, the limitations of this study design prevent reliable estimates of treatment outcomes for efficacy. The study data suggest that fungal infections with Candida, Aspergillus, or Cryptococcus species in patients who were refractory or intolerant to amphotericin B were sometimes successfully treated with AmBisome. However, since the trial was uncontrolled and follow-up was suboptimal,

numerical estimates of clinical and mycological outcome should not be included in a package insert and should not be used for making comparisons with other liposomal formulations. One may conclude that some patients responded to treatment with AmBisome. This is more clear for cryptococcal meningitis, a disease which does not spontaneously resolve. It is less clear for pulmonary aspergillosis or candidemia which may resolve when neutrophil counts are restored or central lines are removed.

FDA assessment of treatment outcome showed numerically lower clinical and mycological response rates when compared to the applicant's analyses. Due to lack of documentation of clinical outcome, both analyses may overestimate actual treatment response. However many of these patients had already "failed" a course of amphotericin B, such that treatment response for primary fungal episodes may be underestimated.

Estimating treatment outcomes in this uncontrolled study was difficult for a number of reasons. First, as in many studies of antifungal drugs, obtaining definitive diagnoses of fungal infections was difficult. MSG diagnostic criteria were not followed during the conduct of the trial. The applicant retrospectively applied diagnostic criteria when analyzing the data. The applicant's definition of definitive infections included definitive, probable or possible infections by MSG criteria.

Second, the quality of the clinical and mycological follow-up was poor. Due to incomplete case report forms, follow-up data on some patients were reconstructed from medical records and discharge summaries. Mycological follow-up was not routinely obtained in a substantial proportion of patients. Mycological response was sometimes considered to be eradicated even if there were no follow-up cultures. The applicant considered the eradication as "implied" based on a successful clinical outcome. Clinical outcome was determined subjectively by the investigator. The submission does not contain data that allows the reviewer to reassess the clinical outcome for every patient based on objective criteria.

With respect to safety, AmBisome appeared to be reasonably well tolerated, However, typical amphotericin B toxicities, such as nephrotoxicity were observed with AmBisome. Increased creatinine levels were observed in approximately 8% of the treated fungal episodes. With no amphotericin B control arm, it is difficult to assess whether common amphotericin B toxicities, such as fever, chills, anemia, hypokalemia and renal insufficiency were reduced by administration of amphotericin B as a liposomal formulation.

There were no reports of allergic or anaphylactoid type reactions, characterized by bronchospasm and dyspnea. Review of events listed as respiratory failure revealed that these were most likely related to underlying disease. However, there was one

report of fatal cardiovascular collapse in a severely ill patient requiring mechanical ventilation for ARDS prior to AmBisome infusion. The investigator did not attribute the fatal event to AmBisome; however assigning relationships of adverse events is very difficult in patients with multiple medical problems.

8.3.2 Protocol 104-10: AmBisome (two dose levels) versus amphotericin B in patients unresponsive to antibiotic therapy for 96 hours or with confirmed fungal infection.

8.3.2.1 Protocol

This protocol studied two types of patients, those with confirmed fungal infections and febrile neutropenic patients who had not responded to antibiotics. In support of an indication for treatment of deep systemic fungal infections, this portion of the review will focus primarily on the confirmed mycosis stratum of the study. Please refer to section 8.1.3 for a review of the empirical antifungal stratum.

8.3.2.1.1 Objectives

The objectives of this trial were to compare the safety and efficacy of two dose levels of AmBisome (1 mg/kg/day and 3 mg/kg/day) with that of conventional amphotericin B in the empiric therapy of fungal infections in neutropenic patients with fever (ET) and in patients with confirmed mycosis (CM).

8.3.2.1.2 Study Design

This study was a prospective, randomized, open-label, parallel, multicenter trial comparing two doses of AmBisome with conventional amphotericin B for empirical antifungal therapy (ET stratum) and for treatment of confirmed mycoses (CM stratum). Patients were randomized within strata to one of the following treatment regimens:

AmBisome 1 mg/kg/day
AmBisome 3 mg/kg/day
Amphotericin B 1 mg/kg/day (dose titrated)

Comment:
The protocol states that the dose of conventional amphotericin B, 1mg/kg/day was chosen to reflect clinical practice. In the U.S. this dose is probably higher than the typical dose used in clinical practice.

8.3.2.1.3 Study Population

The target study size was 200 patients. Inclusion and exclusion criteria for enrollment in the CM stratum are listed below.

Inclusion criteria

Patients greater than 16 years of age and with a serum creatinine < 2 times the upper limit of normal (ULN) or a creatinine clearance > 50 mL/min were eligible if they fulfilled either of the following criteria:

Presence of a confirmed mycosis (CM stratum) with a positive fungal culture or

histologic confirmation by biopsy from an appropriate site.

Antifungal prophylaxis with orally absorbed agents was permitted prior to study enrollment; however, these agents were to be discontinued at randomization.

Exclusion criteria
Patients were excluded from participating if they had a confirmed mycosis that had been treated with systemic antifungal therapy before enrollment.

8.3.2.1.4 Study Procedures
AmBisome was administered intravenously over one hour. Amphotericin B was infused over 4-6 hours using a dose escalation scheme: 0.3 mg/kg was given on day 1, 0.6 mg/kg was given on day 2, and 1.0 mg/kg was given on subsequent days.

Dose reductions of 50% were allowed for patients who experienced nephrotoxicity (defined as a 100% increase from baseline creatinine).

Patients were not to receive any systemic antifungal drug other than randomized study drug. Randomization assignments were held in sealed envelopes and maintained by a person independent of the study. The envelopes were opened sequentially as each patient was enrolled in the study.

Comment:
The procedures for randomization rely on the ability of a person "independent from the study" to control the randomization envelopes. For the pediatric empirical therapy protocol (104-14), this system may have lacked safeguards for preventing protocol violations. In that protocol, one investigator who had access to the envelopes sequentially opened envelopes until the desired treatment regimen was obtained.

Study Assessments
The protocol did not specify the frequency of vital signs measurements. Chemistry and hematology laboratories were to be drawn three times a week. Cultures and biopsies for histologic confirmation were to be obtained when "appropriate".

Comment:
The lack of specific protocol procedures for the assessment of endpoints and the confirmation of fungal infections was a weakness in the study design.

8.3.2.1.5 Endpoints
The protocol contains vague statements regarding the evaluation of efficacy. It states "parameters to be followed and analyzed include the total duration of antifungal therapy, duration of hospitalization, and overall survival." The protocol did not

specifically list clinical or mycological cure rate (for CM stratum) as study endpoints.

Comment:
In the NDA, the applicant more clearly defined a study endpoint. It is not clear whether this was decided prior to the completion of the study. The NDA did not include documentation of protocol amendments for changes in statistical analyses.

For patients in the CM stratum both clinical and mycological outcomes were assessed. Clinical cure was defined as a complete resolution of the patient's signs and symptoms. Mycological cure required repeat negative cultures from a previously positive site.

8.3.2.1.6 Statistical Considerations

According to the protocol, a target sample size of 200 patients (for both strata combined), approximately 67 per arm was based on the ability to detect differences in "serious toxicities" between amphotericin B and AmBisome. Sample size was not based on the ability to detect differences in efficacy or to establish equivalence for the efficacy endpoints.

8.3.2.2 Results (CM Stratum)

The study was conducted from 1992 to 1994 at 18 clinical centers. Although not stated in the original protocol version, patients randomized to amphotericin B were permitted to crossover to AmBisome for toxicity or treatment failure. Crossovers in the other direction were not permitted.

8.1.2.2.1 Patient Disposition

Fifty-nine patients were randomized within the CM stratum and were included in the safety analysis; however, only 39 patients (41 fungal infections)[1] are included in the efficacy analysis. The remaining 20 patients, approximately one-third, were excluded from the efficacy analysis of the CM stratum because they did not meet the definition of a confirmed mycosis.

Six patients switched from amphotericin B to AmBisome. The reason for cross-over was nephrotoxicity for all 6 patients.

Patient #02007 was randomized to receive AmBisome 1 mg/kg/day according to the randomization envelope. The patient actually received 3 mg/kg/day. The applicant analyzed this patient for safety with the 1 mg/kg group and for efficacy with the 3 mg/kg group.

[1] Patients #07009 and #15006 had dual infections with Candidia and Aspergillus species

Table 8.3.6 shows reasons for discontinuation of study drug among patients enrolled in the CM stratum.

Table 8.3.6 CM Stratum: Reasons for Discontinuation of Study drug. Number (percentage)

	Amphotericin B	AmBisome 1 mg/kg	AmBisome 3 mg/kg
Number	20	21	18
Afebrile	1 (5)	3 (14)	1 (6)
Confirmed Mycosis Treated	7 (35)	9 (43)	4 (22)
Severe Adverse Event	0	0	2 (11)
Death	4 (20)	7 (33)	3 (17)
Crossover	6 (30)	NA	NA
Investigator's decision/patient request/other*	2 (10)	2 (10)	8 (44)
treatment success	0	0	2
clinical improvement	0	1	4
treatment failure	0	0	1
toxicity	0	0	1
miscellaneous	2	1	0

Source: Volume 8.1 Table 14.1.2, page 51.

*The applicant further categorized the reason for study drug discontinuation according to what the investigator recorded in the CRF.

8.1.2.2.2 Demographics, comparability

The two strata, ET (empiric therapy) and CM, were different from each other with respect to the prevalence of baseline neutropenia (92% vs. 26% for the ET and CM strata, respectively), fever (90% vs. 48%) and underlying hematologic malignancies (83% vs. 32%). More patients in the CM stratum, compared to the ET stratum, had elevated creatinine and alkaline phosphatase levels at baseline.

Table 8.3.7 shows demographics and baseline characteristics of the patients enrolled in the CM stratum. There were more females randomized to the AmBisome arms and a higher percentage of patients with AML for the amphotericin B arm.

Table 8.3.7. Demographic and Baseline Characteristics.

	Amphotericin B	AmBisome 1 mg/kg	AmBisome 3 mg/kg
Number	20	21	18
Sex			
Male	80%	62%	56%
Female	20%	38%	44%
Race (percent Caucasian)	100%	86%	93%
Age (mean)	55	46	43
Weight (mean)	63	69	68
Primary Diagnosis			
Acute Lymphoblastic Leukemia	5%	10%	0
Acute Myeloid Leukemia	26%	15%	11%
Non Hodgkin Lymphoma	0	15%	11%
Organ transplant	21%	15	17%
Other	47%	45%	61%
Pretreatment Temperature			
mean	38.3	38.0	38.0
median	38.6	37.8	37.9
Pretreatment Serum Creatinine (mmol/L)			
mean	79	96	103
median	65	92	94
Pretreatment Neutrophils			
<500 cells/mm^3	13%	31%	15%

Source: Volume 8.1 Tables 14.1.4, 14.1.6, 14.1.14, 14.1.19, 14.1.24 on pages 53, 54, 62, 69, 76, respectively.

8.1.2.2.3 Efficacy

Table 8.3.8 shows clinical outcome by treatment group and fungal infection for the CM stratum as assessed by the applicant. Table 8.3.9 shows the same for mycological outcome. For the CM stratum, there were small numbers of patients in each of the three treatment arms. When broken down by fungal infection type, essentially infections with Aspergillus species and Candida species, the number of patients for comparisons was further reduced.

Comment:

The following table was reconstructed from the applicant's listing of clinical response. A summary table of clinical response included in the same submission contained slightly different results. I can not account for the discrepancies. Given the small number of

patients with confirmed infections, we did not feel it was worthwhile to re-evaluate clinical or mycological outcomes. Since the data consists of such small numbers, it is unsuitable for display in a package insert. However the data does provide additional support for the antifungal activity of AmBisome.

In general, the treatment arms appeared to be fairly similar for clinical and mycological response. There was no evidence that either dose of AmBisome was clearly worse than amphotericin B. However, the study is grossly underpowered to conclude equivalence.

Table 8.3.8. Clinical Outcome, CM Stratum

	Amphotericin B	AmBisome 1 mg/kg	AmBisome 3 mg/kg
Applicant's Evaluation Total	18*	13	10
All fungal infections			
Cure	7 (39%)	5 (39%)	5 (50%)
Improved	2 (13%)	1 (8%)	1 (10%)
Fail	9 (56%)	6 (46%)	1 (10%)
Not evaluable	1 (6%)	1 (8%)	3 (30%)
Candida Species			
Cure	5	5	4
Improved	0	0	0
Fail	3	4	0
Not evaluable	1	0	2
Aspergillus species**			
Cure	2	0	1
Improved	2	1	1
Fail	5	2	1
Not evaluable	0	1	1

Source: Reconstructed from Vol 8.1, page 82-84, Table 14.2.6
*Two patients had simultaneous infections with Candida species and Aspergillus species
**One infection was with Rhizopus species

Table 8.3.9 Mycological outcome

	Amphotericin B	AmBisome 1 mg/kg	AmBisome 3 mg/kg
Applicant's Evaluation Total	18*	13	10
All fungal infections			
Eradicated	6 (33%)	6 (46%)	5 (50%)
Persistent	9 (50%)	6 (46%)	1 (10%)
Not evaluable	3 (17%)	1 (8%)	4 (40%)
Candida Species**			
Eradicated	5	5	4
Persistent	3	3	0
Not evaluable	1	1	2
Aspergillus species***			
Eradicated	1	1	1
Persistent	6	3	1
Not evaluable	2	0	2

Source: Reconstructed from Vol 8.1, page 82-84, Table 14.2.6
*Two patients had simultaneous infections with Candida species and Aspergillus species
**Includes Torulopsis species
***One infection was with Rhizopus species

8.3.2.2.4 Safety

Table 8.3.10 shows the duration of antifungal therapy and the duration of initially randomized therapy for each of the treatment regimens. The mean duration of antifungal therapy was longest for patients receiving amphotericin B compared to AmBisome. This difference was less apparent when only the initially randomized therapy was considered. This is a reflection of the cross-over option for those randomized to amphotericin B

Table 8.3.10 Mean Duration of treatment and dosage per study arm

	Amphotericin B	AmBisome 1 mg/kg	AmBisome 3 mg/kg
Mean overall treatment antifungal treatment duration	20.5	16.3	11.1
Mean duration of initial therapy	14.8	16.3	11.1
Mean daily dose of initial treatment	0.95	1.12	2.83

Source: Vol. 8.1, page 89, Table 14.3.2

Table 8.3.11 shows the number of patients experiencing any adverse event or any related adverse event and also lists the most common adverse events (those occurring

in at least two patients in any dosing group). There was a larger percentage of patients experiencing adverse events and related adverse events for those randomized to amphotericin B compared to those randomized to AmBisome.

Table 8.3.11 Most prevalent Adverse Events, occurring in two or more patients in at least one group.
Number (Percentage)

	Amphotericin B	AmBisome 1 mg/kg	AmBisome 3 mg/kg
Total	21	21	18
Any Adverse Event	20 (100)	15 (71)	16 (89)
Any related Adverse Event	18 (90)	10 (48)	9 (50)
Body as a Whole			
Chest Pain Substernal	0	2	0
Rigors	3	0	1
Sepsis	1	2	2
Digestive			
Diarrhea	1	2	0
Hepatic Function Abnormal	2	0	0
Nausea	1	1	3
Vomiting	2	2	2
Metabolic and Nutritional			
Alkaline Phos Increased	2	3	1
BUN increased	3	0	0
Bilirubinemia	8	3	1
Hypokalemia	8	0	0
Creatinine Increased	7	1	1
Respiratory			
Pulmonary Edema	2	1	0
Skin			
Rash	0	2	0
Urogenital			
Renal Function Abnormal	2	2	0
Toxic Nephropathy	6	4	3

Source: Vol. 8.1 Page 93 Table 14.3.5

8.3.2.2.5 Overall Conclusions

This study had an unusual design in that it combined the evaluation of empirical therapy and treatment of confirmed mycoses. Since different endpoints are required to assess the efficacy of these treatment indications, separate studies with sufficient power to

detect differences in treatment arms would have been preferable. Due to the small number of patients with definitively confirmed mycoses in this study, one is unable to form any conclusions regarding the comparative efficacy between AmBisome and amphotericin B. A number of patients treated with AmBisome appeared to have resolution of their pretreatment fungal infections. It did not appear that either dose of AmBisome was worse than amphotericin B, but there is clearly a lack of statistical power to imply equivalence.

In addition, patients initially assigned to amphotericin B were allowed to switch to AmBisome for toxicity or failure. This unidirectional cross-over procedure would be predicted to add systematic bias to the analysis. The overall effect of this potential bias may be expected to make treatment groups look more similar.

AmBisome appeared to be somewhat better tolerated than amphotericin B. There were less "related" adverse events among patients receiving AmBisome. However, this was an open-label study so that the classification of drug relationship may have been biased. Please refer to section 8.1.3 for a more detailed safety review of this study.

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ON ORIGINAL

8.3.3 Study No. 104-05: AmBisome vs. Conventional Amphotericin B for Confirmed Deep Fungal Infections in Patients with Neutropenia

8.3.3.1 Protocol

8.3.3.1.1 Objectives

To compare the safety and clinical and mycological efficacy of AmBisome and conventional amphotericin B for the treatment of confirmed deep fungal infections in patients with neutropenia.

8.3.3.1.2 Study Design

The study was a multicenter, randomized, open-label trial in which neutropenic patients with deep fungal infections were randomized (1:1) to one of the following treatment arms:

AmBisome 5 mg/kg (days 1-14), 3 mg/kg (days 15-28)
Amphotericin B (1 mg/kg for Aspergillus species)

The protocol also stated that treatment was to continue for 14-28 days or until all previously positive cultures became negative and the signs and symptoms of fungal infection were resolved. Continuation of treatment or "maintenance" treatment after completion of the study was permitted but not considered part of the protocol. Concomitant use of other systemic antifungals including 5-FC (Fluorocytosine) was not allowed.

8.3.3.1.3 Study Population

Protocol inclusion criteria required that patients be hospitalized with clinical evidence of deep fungal infection or have evidence of a fungal infection within 2 weeks of an episode of severe neutropenia (<0.5 X 10^9 neutrophils).

The protocol stated that all efforts for mycological confirmation were to be made, but patients could be enrolled pending culture results if invasive aspergillosis or candidiasis was probable.

Enrollment criteria

The following were criteria the investigators considered when enrolling patients. They were not criteria for classifying infections as definitive or presumptive.

For invasive candidiasis and other yeast infections:

- Confirmation of yeast by microscopic examination of deep tissue specimens.
- Positive identification of Candida species using at least one culture of blood or material from deep tissue sites or from body spaces that are normally sterile.

Positive cultures of respiratory secretions alone did not confirm invasive candidiasis.

- The presence of fever, plus no response to antibacterial therapy, plus positive Candida antigen test of serum or the presence of specific signs as endophthalmitis, skin lesions or visceral foci. For these patients, if pretreatment cultures remained negative after 14 days patients were to be withdrawn form the study.

Comment:
Antigen serologies for Candida species are not considered diagnostic for invasive candidiasis by MSG criteria.

For invasive aspergillosis and other non-yeasts:

- Confirmation by positive identification of fungi by microscopic examination of deep tissue specimens or respiratory secretions plus, abnormal radiographs without evidence of other pathogens or,
- The presence of pulmonary infiltrates not responding to 5 days of antibacterial therapy.

Exclusion criteria
Patients were excluded from participating if they were under age 16, HIV-infected, or had a serum creatinine in excess of 250 µmol. In addition, the use of prophylactic systemic antifungal drugs was only permitted if these drugs had been used for at least 7 days before any symptoms of infection were apparent. According to the protocol this indicated that the fungal infection had emerged despite prophylaxis.

8.3.3.1.4 Study Procedures

Investigators were to record vital signs, signs and symptoms of fungal infection and to obtain serum for determination of BUN, creatinine and potassium on a daily basis. Every seven days, radiographs (if previously abnormal) were to be repeated. Culture and microscopic examination of previously positive sites, and hematology and chemistry lab tests were to be repeated every 7 days. Post-treatment evaluations were to be completed 48 hours and 2-4 weeks after discontinuation of drug.

The protocol required investigators to classify the severity of the fungal infection at baseline as follows:

For Pulmonary Aspergillosis

Mild	only fever and radiographic abnormalities, no dyspnea
Moderate	mild or moderate dyspnea and/or bilateral and extensive radiographic abnormalities

Severe	severe dyspnea (pO_2 < 60 mm Hg)
Very Severe	mechanical ventilation required

<u>For systemic candidiasis or disseminated aspergillosis</u>

Mild	only fever or mild illness without specific signs
Moderate	localized mucocutaneous, ophthalmic, or organic lesions
Severe	widespread metastatic lesions
Very Severe	hypotension or coma

Comment:
This classification system appears to be reasonable; however, it is unknown if there is significant prognostic differences between all categories.

8.3.3.1.5 Endpoints

The clinical efficacy of the study drugs were to be determined by the rate of success or improvement after 14 days of therapy, where success was defined as the elimination of pretreatment signs and symptoms with progressive radiographic improvement of disease. Clinical improvement was defined as a reduction, but not complete elimination, of signs and symptoms. Secondary clinical endpoints included the rate of clinical success after 28 days, the time to success, the survival rate and the rate of clinical relapse.

The primary mycological endpoint was the rate of eradication of the pathogen after 14 days of therapy.

For comparison of safety data, a 100% increase in serum creatinine was considered to be of interest. Earlier versions of the protocol specified a 50% increase, this was later amended to 100%.

8.3.3.1.6 Statistical Considerations

The target enrollment for this protocol was 60 patients. This sample size was based on the ability to have 90% power, with a 5% level of significance, to show a 40% difference in the rate of clinical "improvement" (assuming 80% for AmBisome vs. 40% for amphotericin B).

Comment:
This study was designed with statistical power to detect a 100% improvement in the rate of clinical success. It would have been preferable to determine sample size based on the ability to detect smaller degrees of improvement for success rates. In addition the sample size calculations did not take into consideration the loss of patients due to inability to confirm invasive fungal infections.