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# Safety, Tolerability, and Pharmacokinetics of Liposomal Amphotericin B in Immunocompromised Pediatric Patients

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**ABSTRACT** The safety, tolerability, and pharmacokinetics of the liposomal formulation of amphotericin B (L-AMB) were evaluated in 40 immunocompromised children and adolescents. The protocol was an open-label, sequential-dose-escalation, multidose pharmacokinetic study with 10 to 13 patients in each of the four dosage cohorts. Each cohort received daily dosages of 2.5, 5.0, 7.5, or 10 mg of amphotericin B in the form of L-AMB per kg of body weight. Neutropenic patients between the ages of 1 and 17 years were enrolled to receive empirical antifungal therapy or treatment of documented invasive fungal infections. The pharmacokinetic parameters of L-AMB were measured as those of amphotericin B by high-performance liquid chromatography and calculated by noncompartmental methods. There were nine adverse-event-related discontinuations, four of which were related to infusions. Infusion-related side effects occurred for 63 (11%) of 565 infusions, with 5 patients experiencing acute infusion-related reactions (7.5- and 10-mg/kg dosage levels). Serum creatinine levels increased from 0.45 ± 0.04 mg/dl to 0.63 ± 0.06 mg/dl in the overall population ($P = 0.003$), with significant increases in dosage cohorts receiving 5.0 and 10 mg/kg/day. At the higher dosage level of 10 mg/kg, there was a trend toward greater hypokalemia and vomiting. The area under the concentration-time curve from 0 to 24 h ($AUC_{0-24}$) values for L-AMB on day 1 increased from 54.7 ± 32.9 to 430 ± 566 $\mu g \cdot h/ml$ in patients receiving 2.5 and 10.0 mg/kg/day, respectively. These findings demonstrate that L-AMB can be administered to pediatric patients at dosages similar to those for adults and that azotemia may develop, especially in those receiving ≥5.0 mg/kg/day.

**KEYWORDS** antimicrobial safety and tolerability, hematological malignancies, liposomal amphotericin B, pediatrics, pharmacokinetics

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Invasive fungal infections are important causes of morbidity and mortality in neutropenic pediatric patients (1–6). Liposomal amphotericin B (L-AMB) has been used for prophylaxis, empirical antifungal therapy, and treatment of documented mycoses in children and adults (7–15). However, little is known about the pharmacokinetic prop-

**TABLE 1** Demographic features of enrollees by dosage group[a]

| Parameter | Value for treatment group | | | |
|---|---|---|---|---|
| | 2.5 mg/kg ($n$ = 10) | 5.0 mg/kg ($n$ = 13) | 7.5 mg/kg ($n$ = 12) | 10 mg/kg ($n$ = 12) |
| Mean age (yr) ± SD | 6.8 ± 4.9 | 7.7 ± 4.0 | 8.3 ± 6.2 | 8.2 ± 5.1 |
| No. of male patients:no. of female patients | 7:3 | 9:4 | 8:4 | 8:4 |
| No. of patients with reason for enrollment | | | | |
| Empirical antifungal therapy | 10 | 9 | 11 | 10 |
| Documented invasive fungal infection | 0 | 4[b] | 1[b] | 0 |
| Median duration of study drug (days) (range) | 14.5 (3–29) | 12 (2–27) | 5 (1–33) | 7.5 (1–42) |

[a]There were 40 patients, with 47 enrollments, in this study. Two patients in the 5-mg/kg group and 3 patients in the 7.5-mg/kg group were previously enrolled in the lower-dosage group. Two patients in the 10-mg/kg group were previously enrolled in the group with the same dosage level of 10 mg/kg.
[b]Four patients enrolled in the 5-mg/kg dosage cohort had documented invasive fungal infections consisting of one case each of fungemia due to *Candida albicans* and *C. parapsilosis*, one case of pulmonary aspergillosis due to *Aspergillus fumigatus*, and one case of cryptococcal meningitis. One patient in the 7.5-mg/kg cohort had documented candidal pneumonia due to *C. albicans*.

erties of liposomal amphotericin B in pediatric patients (9, 16). To our knowledge, only one trial has reported the pharmacokinetics of multiple dosages of this compound in children (16). We studied the safety, tolerability, and pharmacokinetics of L-AMB administered as empirical antifungal therapy to persistently febrile neutropenic pediatric patients in a sequential-dose-escalation, multidose pharmacokinetic study.

## RESULTS

**Study patient demographics.** A total of 40 patients, with 47 enrollments in the study, received at least one dose of L-AMB (Table 1). Five patients were enrolled more than one time. Thirty-six patient enrollees received at least three doses of L-AMB. These patient enrollees (32 males and 15 females) had a mean age of 7.8 years. Underlying conditions included antineoplastic chemotherapy (31 patients), allogeneic progenitor (hematopoietic) stem cell transplantation (PSCT) (10), HIV infection (4), aplastic anemia (1), and chronic granulomatous disease (1).

**Safety.** All but three patients experienced at least one adverse event during the study (Table 2). No consistent dosage-related trend in most adverse events was observed. However, at the higher dosage level of 10 mg/kg of body weight, there was a trend toward greater hypokalemia and vomiting.

The differences in serum creatinine levels at baseline and the end of therapy by dosage cohort are summarized in Table 3. Patients in the 5.0-mg/kg and 10-mg/kg cohorts had significant increases in mean serum creatinine levels during the course of

**TABLE 2** Common adverse events with liposomal amphotericin B by dosage group[a]

| Adverse event | No. of patients with adverse event in cohort | | | |
|---|---|---|---|---|
| | 2.5 mg/kg ($n$ = 10) | 5.0 mg/kg ($n$ = 13) | 7.5 mg/kg ($n$ = 12) | 10 mg/kg ($n$ = 12) |
| Total | 9 | 12 | 10 | 12 |
| Abdominal pain | 4 | 2 | 2 | 4 |
| Fever | 3 | 5 | 2 | 4 |
| Chills | 0 | 4 | 2 | 4 |
| Diarrhea | 1 | 4 | 4 | 3 |
| Vomiting | 2 | 2 | 1 | 6 |
| Dyspnea | 4 | 1 | 6 | 5 |
| Cough | 1 | 3 | 3 | 3 |
| Rash | 0 | 3 | 4 | 4 |
| Hypokalemia (<3 meq/liter) | 4 | 4 | 2 | 8 |
| Hyperglycemia | 1 | 2 | 1 | 5 |
| Elevated BUN level | 0 | 2 | 1 | 4 |

[a]There were 40 patients with 47 enrollments in this study. Two patients in the 5-mg/kg group and 3 patients in the 7.5 mg/kg-group were previously enrolled in the lower-dosage group. Two patients in the 10-mg/kg group were previously enrolled in the group with the same dosage level of 10 mg/kg.

**TABLE 3** Mean serum creatinine levels by liposomal amphotericin B dosage group

| Dosage of L-AMB (mg/kg) (no. of patients) | Mean serum creatinine level (mg/dl) ± SEM | | *P* value[a] |
|---|---|---|---|
| | Baseline | End of therapy | |
| 2.5 (10) | 0.33 ± 0.01 | 0.49 ± 0.12 | 0.19 |
| 5.0 (13) | 0.44 ± 0.04 | 0.81 ± 0.14 | 0.001 |
| 7.5 (12) | 0.64 ± 0.13 | 0.62 ± 0.10 | 0.9 |
| 10 (12) | 0.37 ± 0.04 | 0.54 ± 0.09 | 0.04 |

[a]As determined by a Wilcoxon rank sum test.

antifungal therapy. When the changes from the baseline to the end of therapy were analyzed for all patients, serum creatinine levels increased from 0.45 ± 0.04 mg/dl to 0.63 ± 0.06 mg/dl ($P = 0.003$).

The changes in serum creatinine levels at baseline and the end of therapy in individual patients are depicted in Fig. 1. There was no consistent pattern between the dosage cohort and the number of patients with an increase in the serum creatinine level or in the magnitude of the change in the serum creatinine levels in individual subjects. These findings suggest that considerable interpatient variation occurred in the predilection for LAMB-related nephrotoxicity.

Two patients were withdrawn from the study for laboratory abnormalities. One patient had an elevated serum creatinine level, and another patient had increased hepatic transaminase levels; both patients were enrolled in the 5-mg/kg dosage cohort. The study drug was discontinued in the former patient because of a rapid rise in serum creatinine levels, which increased from 0.5 mg/dl on day 22 to 1.7 mg/dl on day 26.

**Tolerability.** All infusions of L-AMB were directly monitored; vital signs and symptoms were recorded in a data collection sheet at the patient's bedside. There was no consistent dose-related trend observed with respect to the overall frequency of infusion-related reactions (IRRs) (Table 4). Three (25%) of 12 patients in the 10-mg/kg

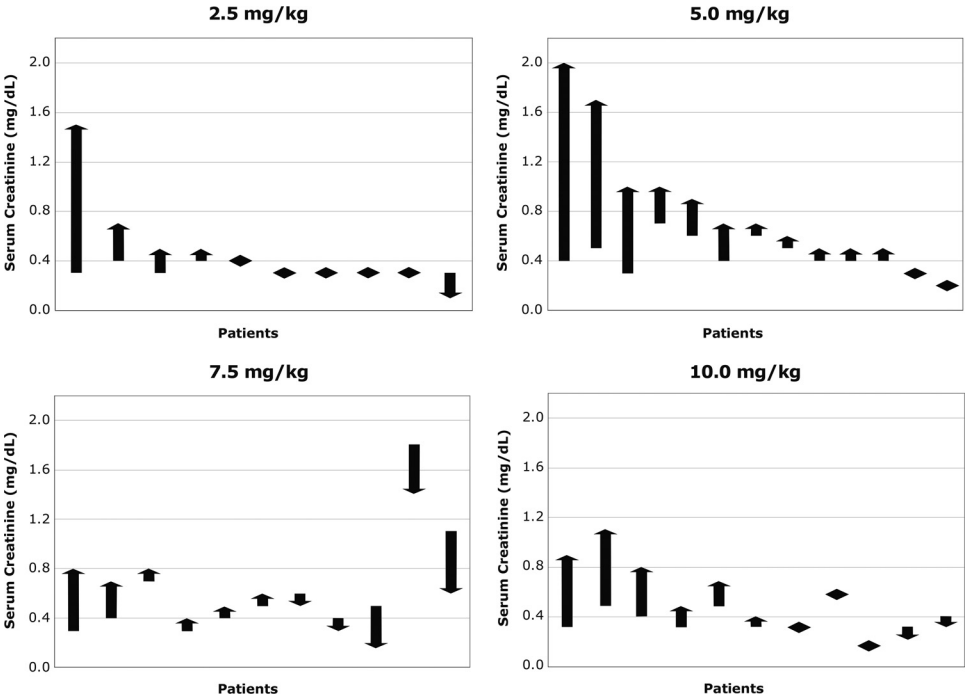


**FIG 1** Changes in serum creatinine levels in individual pediatric patients receiving liposomal amphotericin B at 2.5, 5.0, 7.5, and 10.0 mg/kg/day. Arrows indicate the direction of the change of serum creatinine levels from the baseline to the end of therapy. The origin of the arrow indicates the serum creatinine value at baseline, while the tip of the arrow indicates the serum creatinine value at the end of therapy. Data for those patients with no change in serum creatinine levels between the baseline and the end of therapy are indicated by ◆.

**TABLE 4** Infusion-related reactions with liposomal amphotericin B by dosage group

| Parameter | Value for dosage group | | | |
|---|---|---|---|---|
| | 2.5 mg/kg ($n$ = 10) | 5.0 mg/kg ($n$ = 13) | 7.5 mg/kg ($n$ = 12) | 10 mg/kg ($n$ = 12) |
| No. of patients with IRR | | | | |
| Total | 4 | 5 | 6 | 6 |
| Fever (temp ≥ 1°C) | 3 | 2 | 1 | 0 |
| Chills/rigors | 0 | 1 | 1 | 3 |
| Nausea | 0 | 0 | 0 | 1 |
| Vomiting | 0 | 0 | 0 | 3 |
| Dyspnea | 0 | 0 | 4 | 2 |
| Chest pain | 2 | 0 | 1 | 0 |
| Abdominal pain | 1 | 0 | 0 | 1 |
| Facial flushing | 0 | 1 | 1 | 2 |
| Pruritis or urticaria | 0 | 1 | 0 | 1 |
| No. of infusions | 148 | 173 | 101 | 143 |
| No. of IRR episodes | 10 | 16 | 16 | 21 |
| No. of IRR episodes for which patients received treatment[a] | 3 | 5 | 5 | 5 |
| No. of patients discontinuing study drug for IRRs | 0 | 0 | 3 | 2 |

[a]The following medications were used for treatment of L-AMB-associated IRRs: acetaminophen ($n$ = 11), diphenhydramine ($n$ = 11), hydroxyzine ($n$ = 1), methylprednisolone ($n$ = 1), pethidine ($n$ = 2), and promethazine ($n$ = 1).

dosage cohort reported chills/rigors and vomiting. Chills and/or rigors were present for 5 (11%) of the day 1 infusions. Among the IRRs, a cluster of symptoms, including facial flushing, pruritus, dyspnea, chest pain, and abdominal pains, occurred in 10 episodes, suggesting a histamine-mediated reaction. In addition, musculoskeletal symptoms such as myalgias and arthralgias were also reported.

There were 63 episodes of IRRs (11%) among the 565 infusions of L-AMB. Eighteen patients received treatment for infusion-related side effects. Five patients discontinued the study drug due to IRRs. There was no relationship among the dosage group, frequency of IRRs, or discontinuation due to IRRs.

**Pharmacokinetics.** The amphotericin B pharmacokinetic parameters calculated by using noncompartmental analysis are summarized in Tables 5 and 6 for the first and last days, respectively. The concentration-time curves for the first- and last-day pharmacokinetics are presented in Fig. 2 and 3, respectively. There was a high degree of interpatient variability in pharmacokinetic parameters for the first and last days. The mean area under the concentration-time curve to infinity ($AUC_{inf}$) values on day 1 were higher than anticipated for the 5- and 10-mg/kg dosages and lower than expected for the 7.5-mg/kg dosage, consistent with nonlinear pharmacokinetics (Fig. 2). The mean $AUC_{inf}$ values on the last day of infusion were higher than expected for the 5-mg/kg dosage and lower than anticipated for the 7.5-mg/kg and 10-mg/kg dosages (Fig. 3).

**TABLE 5** Pharmacokinetic parameters of liposomal amphotericin B in pediatric patients on day 1 by dosage group

| Parameter | Value for dosage group[b] | | | |
|---|---|---|---|---|
| | 2.5 mg/kg ($n$ = 10) | 5.0 mg/kg ($n$ = 13) | 7.5 mg/kg ($n$ = 8)[a] | 10 mg/kg ($n$ = 8)[a] |
| Mean $AUC_{0-24}$ ($\mu$g/ml · h) ± SD | 54.7 ± 32.9 | 351 ± 445 | 134 ± 80.9 | 430 ± 566 |
| Mean $AUC_{0-\infty}$ ($\mu$g/ml · h) ± SD | 75.7 ± 31.9 B | 442 ± 551 | 168 ± 95.0 | 548 ± 604 B |
| Mean $C_{max}$ ($\mu$g/ml) ± SD | 15.1 ± 9.0 C | 46.2 ± 46.7 | 30.0 ± 20.5 | 67.9 ± 74.2 C |
| Mean $t_{1/2}$ (h) ± SD | 8.8 ± 2.1 | 12.6 ± 8.4 | 13.5 ± 8.6 | 8.7 ± 3.8 |
| Mean $V$ (liters/kg) ± SD | 0.47 ± 0.18 | 0.86 ± 0.86 | 1.22 ± 1.37 | 0.68 ± 0.90 |
| Mean CL (ml/h/kg) ± SD | 38 ± 13 | 45 ± 38 | 60 ± 38 | 46 ± 39 |

[a]Serum samples from 4 patients each in the 7.5-mg/kg and 10-mg/kg dosage cohorts were not available for analysis.

[b]B and C indicate $P$ values of $<0.05$ for comparisons.

**TABLE 6** Pharmacokinetic parameters of liposomal amphotericin B on the last day by dosage group

| Parameter | Value for dosage group[a] | | | |
|---|---|---|---|---|
| | 2.5 mg/kg ($n$ = 4) | 5.0 mg/kg ($n$ = 5) | 7.5 mg/kg ($n$ = 3) | 10 mg/kg ($n$ = 5) |
| Mean $AUC_{0-24}$ ($\mu$g/ml · h) ± SD | 301 ± 180 | 767 ± 1,115 | 395 ± 216 | 786 ± 689 |
| Mean $AUC_{0-\infty}$ ($\mu$g/ml · h) ± SD | 327 ± 194 | 821 ± 1,118 | 482 ± 167 | 857 ± 696 |
| Mean $C_{max}$ ($\mu$g/ml) ± SD | 49.5 ± 31.9 | 64.1 ± 45.8 | 57.4 ± 24.0 | 83.1 ± 48.9 |
| Mean $t_{1/2}$ (h) ± SD | 14.3 ± 7.5 | 17.9 ± 4.8 | 21.3 ± 8.8 | 27.4 ± 23.5 |
| Mean $V$ (liters/kg) ± SD | 0.20 ± 0.16 | 0.31 ± 0.20 | 0.53 ± 0.35 | 0.63 ± 0.51 |
| Mean CL (ml/h/kg) ± SD | 10 ± 6 | 13 ± 12 | 17 ± 7 | 17 ± 11 |

[a]Serum samples from 8 patients in the 2.5-mg/kg dosage cohort, 8 in the 5.0-mg/kg cohort, 9 in the 7.5-mg/kg cohort, and 7 in the 10-mg/kg cohort were not available for analysis.

Similar trends were observed for the maximum concentration of drug in serum ($C_{max}$) for the 5.0-mg/kg dosage and the 7.5-mg/kg dosage. The mean $AUC_{inf}$ for each dosage cohort tended to increase between the first day and the last day.

The mean clearance (CL) value appeared to increase with increasing dosages on both the first and last days, with the exception of the 10-mg/kg dosage on the first day. Clearance was consistently diminished after multiple dosing in comparisons between the first and last days in all dosage cohorts, suggesting a saturable process. The volume of distribution ($V$) was determined to be <1.0 liters/kg for most dosages on the first and last days.

**Outcome and efficacy.** Measures of overall antifungal efficacy and outcome by dosage cohort are presented in Table 7. Twenty-seven (75%) of the 36 enrollments in which patients received at least three doses of the study drug were considered to have a successful outcome for empirical therapy or for treatment of documented infection. Fourteen (39%) of these 36 enrollments resulted in the complete resolution of all signs and symptoms.

Two patients at the 2.5-mg/kg dosage level developed breakthrough infections (pulmonary aspergillosis and blood culture positive for *Candida parapsilosis*). Three failures were observed in the 5-mg/kg dosage group. One patient died from graft-versus-host disease, increased creatinine levels, and rash. A patient with chronic granulomatous disease developed pneumonia due to a filamentous fungal pathogen. A PSCT recipient discontinued therapy secondary to radiological progression of suspected invasive aspergillosis. Two patients at the 7.5-mg/kg dose level were considered to be outcome failures. One patient developed hyperbilirubinemia, and a patient with Wiskott-Aldrich syndrome and non-Hodgkin's lymphoma had pulmonary aspergillosis diagnosed after 30 days of therapy. There were no outcome failures in the 10-mg/kg cohort; however, there was also no trend of a dose-response relationship in efficacy.

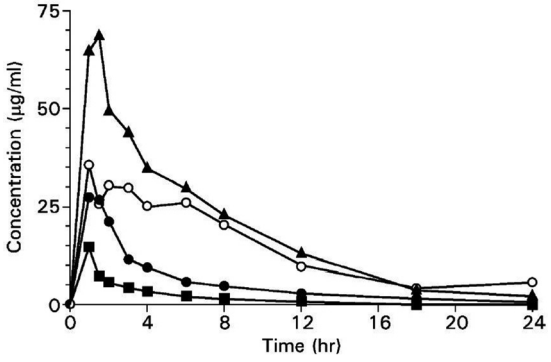


**FIG 2** Concentration-time curves of liposomal amphotericin B in pediatric patients receiving 2.5 mg/kg (■), 5.0 mg/kg (●), 7.5 mg/kg (○), and 10.0 mg/kg (▲) on day 1 of infusion.

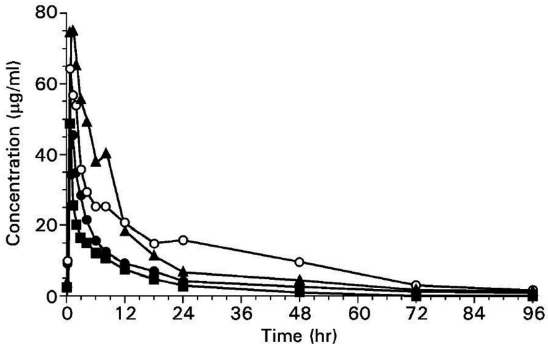


**FIG 3** Concentration-time curves of liposomal amphotericin B in pediatric patients receiving 2.5 mg/kg (■), 5.0 mg/kg (●), 7.5 mg/kg (○), and 10.0 mg/kg (▲) on the last day of infusion.

The rate of survival within the first 4 days posttreatment was 88% (35/40 patients; 89% [42/47] of enrollments). The rate of survival within 2 months posttreatment was 75% (30/40 patients; 79% [37/47] of enrollments). There was no consistent dose-related trend for survival.

## DISCUSSION

This prospective trial of the safety, tolerability, and plasma pharmacokinetics of L-AMB in pediatric patients found no dose-limiting toxicity in the four dosage groups from 2.5 mg/kg/day to 10 mg/kg/day. At the higher dosage levels of 7.5 to 10 mg/kg, there was a trend toward greater hypokalemia and azotemia as well as infusion-related dyspnea, vomiting, chills, and rigors. The pharmacokinetic parameters in pediatric patients were similar to those in adults, indicating that based upon these data, no dosage adjustment for L-AMB is indicated for pediatric patients. Although this study was not designed to assess efficacy, there was no significant dose-response relationship with therapeutic outcome.

The trend in AUCs indicates a pattern of high interpatient variability similar to that for adults in two previous clinical trials. The first trial studied dosages of 1.0 to 7.5

**TABLE 7** Measures of overall antifungal efficacy and outcome by dosage group

| Parameter | Value for dosage group[g] | | | |
|---|---|---|---|---|
| | 2.5 mg/kg ($n$ = 10) | 5.0 mg/kg ($n$ = 13) | 7.5 mg/kg ($n$ = 12) | 10 mg/kg ($n$ = 12) |
| No. (%) of patients surviving at 4 days posttreatment | 8 (80) | 12 (92) | 10 (83) | 12 (100) |
| No. (%) of patients surviving at 2 mo posttreatment | 8 (80) | 11 (85) | 7 (58) | 11 (92) |
| No. (%) of patients with breakthrough invasive fungal infection | 2 (20)[a] | 1 (8)[b] | 1 (8)[c] | 0 |
| No. of patients with baseline invasive fungal infection | 1[d] | 5[e] | 1[f] | 0 |
| No. of patients with successful treatment of baseline invasive fungal infection | 1[d] | 4[e] | 0[f] | 0 |
| Type(s) of successful treatment of baseline invasive fungal infection | Partial response | 2 stabilized, 2 complete response | None | NA |
| Type of failure | | 1 relapse | Progression | NA |

[a]Invasive pulmonary aspergillosis developed in one patient, and *Candida parapsilosis* fungemia developed in another patient in the study.
[b]Filamentous fungal pneumonia (species not identified).
[c]Invasive pulmonary aspergillosis.
[d]Severe fluconazole-resistant esophageal candidiasis with resolution of odynophagia and dysphagia and partial reduction of mucosal plaques.
[e]One patient had relapsed cryptococcal meningitis after 3 weeks of L-AMB and 4 days of maintenance fluconazole. Among all patients with a successful response, two patients had candidemia caused by *C. albicans* and *C. parapsilosis*, which completely resolved, and two patients with invasive pulmonary aspergillosis had stable disease.
[f]One patient died with culture- and biopsy-proven acute pulmonary and hepatic disseminated candidiasis and refractory leukemia after 2 days of L-AMB following withdrawal of all supportive care.
[g]NA, not applicable.

mg/kg (17), and the second trial studied dosages of 7.5 to 15 mg/kg (18). The trends of AUC versus dosage bore some similarity to those for adults but were also distinctive. For example, the mean AUC from time zero to infinity ($AUC_{0-\infty}$) for the 10-mg/kg dose on the last day was ~2.5 times the $AUC_{0-\infty}$ for 2.5 mg/kg, while one would anticipate a 4-fold difference.

A comparison of L-AMB exposures between pediatric patients in this study and adult patients (18) for the mean last-day AUC from time zero to 24 h ($AUC_{0-24}$) and $AUC_{0-\infty}$ values reveal compatible exposures at 2.5, 5.0, and 7.5 mg/kg/day. The differences in the mean last-day $AUC_{0-24}$ and $AUC_{0-\infty}$ values for a dose of 10 mg/kg/day, being higher in adults than in pediatric patients, may be related to interpatient variability. A population pharmacokinetic model, which was developed from these data, demonstrated nonlinear pharmacokinetics as well as a time-dependent change that was not explained by any of the covariates monitored in this study (19).

Among the few other studies that have reported circulating concentrations or pharmacokinetics of L-AMB in children, Mehta and colleagues (9) studied the pharmacokinetics of a once-weekly 10-mg/kg L-AMB dosage in 14 pediatric patients undergoing hematopoietic stem cell transplant. Unfortunately, as these concentrations were reported for non-lipid-complexed amphotericin B, a comparison of results is not feasible. Similarly, Kotwani et al. studied only one dosage level of 1.0 mg/kg over 28 days in adults, children, and neonates (20). This study suggested that neonates may have a larger volume of distribution than those of adults. Hong and colleagues reported a population pharmacokinetic model of L-AMB in pediatric patients with malignant diseases receiving dosages ranging from 0.8 to 5.9 mg/kg/day (16).

The therapeutic implications of dose-dependent plasma exposure in children warrant further study. Indeed, these findings are compatible with data from an adult study that demonstrated no apparent benefit in therapeutic outcomes with higher dosages exceeding 7.5 mg/kg (18) as well as with data from the recent "AmBiload" study of invasive aspergillosis in adults (7). In that study, Cornely and colleagues demonstrated that higher dosages of L-AMB did not result in a higher response rate in a prospective randomized trial of L-AMB comparing dosages of 3.0 mg/kg and 10 mg/kg per day for primary treatment of proven and probable invasive aspergillosis in 201 patients. That study found similar survival rates and overall response rates in both dosage groups but a greater frequency of toxicity in the higher-dosage group.

Among the toxicity profiles seen in this study, there were infusion-related and non-infusion-related adverse events, as reported in case reports and case series (11, 17, 21–26). Although the numbers of patients with overall IRRs were similar in each dosage group (4 to 6 per patient), there was a trend of some IRRs (chills/rigors, vomiting, and dyspnea) to occur in the groups receiving higher dosages of 7.5 to 10.0 mg/kg. Five patients also displayed features of the acute infusion-related reaction complex previously described for adults and adolescents (26), which led to discontinuation. Each of these events occurred with the higher dosages of 7.5 and 10 mg/kg/day. Among the five patients with acute infusion-related reactions (AIRRs), two had dyspnea only; a third had dyspnea plus abdominal pain; a fourth had dyspnea, facial flushing, nausea, and vomiting; and the fifth patient had dyspnea, lip swelling, and facial flushing. Although these events occurred in the higher-dosage group, the AIRRs began early during infusion, and the absolute amount of L-AMB infused was relatively small (6 mg to <50 mg). The possible mechanism for these events may be related to liposomal activation of the C5a component of the complement cascade with ensuing histamine release, as previously described (27).

While pediatric patients are better able to resist the clinically overt nephrotoxicity of amphotericin B, renal impairment remains a dose-limiting toxicity in this population (11). The mean serum creatinine levels in three of the four dosage groups increased by ~60 to 90% from the baseline (Table 4). In understanding the reason for the lack of an appreciable change in mean serum creatinine levels in the 7.5-mg/kg dosage cohort, this group of patients had higher preexisting elevated mean baseline serum creatinine levels (0.64 mg/dl) than did the other three dosage cohorts (mean baseline serum

creatinine levels of 0.33 to 0.44 mg/dl). Even at dosages of 10 mg/kg, the nephrotoxic adverse effects of L-AMB were well tolerated in most patients.

There are several possible mechanisms for renal protection of L-AMB and other lipid formulations of amphotericin B (28): reduced induction of tubuloglomerular feedback (29), high-affinity binding to high-density lipoproteins with decreased renal accumulation (30, 31), selective cytotoxicity to fungal versus mammalian cells (32, 33), reduced toxicity to renal vascular endothelial cell membranes (34), and organism-mediated phospholipase-induced release of amphotericin B from lipid formulations (35). Nevertheless, a 7-year-old HIV-infected patient with relapsed cryptococcal meningitis, after 3 weeks of 5 mg/kg L-AMB, sustained a precipitous rise in serum creatinine levels from 0.5 to 1.7 mg/dl over 4 days, necessitating withdrawal from the study. While double-blind randomized trials demonstrate that administration of L-AMB is significantly less nephrotoxic than conventional deoxycholate amphotericin B (DAmB) (11, 15), close monitoring of the renal function of pediatric patients receiving L-AMB is certainly warranted.

The efficacy of L-AMB has been reported for pediatric patients with a variety of invasive fungal infections (10, 36–42). Although this phase I-II study was not powered to determine therapeutic outcome, several observations bear note. All dosages of L-AMB in this study conferred a therapeutic response in some patients. There was no significant relationship between dosage and frequency of breakthrough invasive fungal infections. Therapeutic responses to baseline invasive fungal infections also did not correlate with response. These findings are compatible with those of the above-described adult studies, which did not demonstrate a dose-response relationship with dosages of 3 mg/kg/day to 10 mg/kg/day for candidiasis and aspergillosis (7, 18). For other organisms, such as the Mucorales, which have higher MICs, dosages of 5 to 10 mg/kg/day may be more effective (43).

The $C_{max}$-to-MIC ($C_{max}$/MIC) ratio is the parameter that is predictive of the outcome of DAmB treatment in experimental murine candidiasis. A $C_{max}$/MIC ratio of 5 to 10 results in a >103-fold reduction in CFU per gram of the residual fungal burden in kidneys (44). Assuming an ~5-fold difference in potency between L-AMB and DAmB against invasive candidiasis, the $C_{max}$/MIC ratio for L-AMB would be 25 to 50. Given an MIC of 0.25 $\mu$g/ml for *Candida albicans*, the $C_{max}$ needed to attain the target ratio is 10 to 20 $\mu$g/ml (45). The data from this study indicate that all studied dosages from 2.5 mg/kg to 10 mg/kg would yield mean $C_{max}$ values that would achieve the target $C_{max}$/MIC ratio of between 25 and 50.

There are several limitations of this clinical trial. Among these limitations is the different numbers of patients with data for the first dose versus the last dose within a given dosage cohort. This reflects the practical limitations of patients being discharged before the last day of pharmacokinetics can be obtained. Yet another limitation is the variation in the duration of antifungal therapy. This variation is understood as a reflection of the different durations of antifungal therapy needed for the management of each patient.

In summary, this study documents the safety, tolerability, and pharmacokinetics of L-AMB in immunocompromised pediatric patients. Infusion-related toxicity, while uncommon, may require discontinuation. The overall relationships between dosage and exposure were similar for children and adults; however, the wide interpatient variability warrants further assessment with population-based pharmacokinetic modeling.

## MATERIALS AND METHODS

**Study design.** The objective of the study was to evaluate the safety, tolerability, and pharmacokinetics of intravenous L-AMB (AmBisome; Astellas Pharma USA, Inc., Deerfield, IL, USA) at four dosage levels in a population of immunocompromised pediatric patients, including those with cancer, PSCT, HIV/AIDS, and other immunodeficiencies. Patients were eligible for the study if (i) they were between the ages of 1 and 17 years, undergoing PSCT, or receiving active chemotherapy for neoplastic disease and (ii) had persistent or recurrent fever (oral temperature of ≥38.0°C) and neutropenia (absolute neutrophil count of <500/$\mu$l) despite broad-spectrum antibacterial therapy for five or more days or had culture- or biopsy-proven invasive fungal infection. No forms of amphotericin B, other than the study drug, were allowed during the study. The protocol was reviewed and approved by each Institutional Review Board

(IRB). The IRB-approved informed consent was obtained from the patient or their legally authorized representative prior to entry.

Patients were not eligible for enrollment into the study if (i) the patient had received deoxycholate or other formulations of amphotericin B within 1 week prior to study entry; (ii) there was clinical and laboratory evidence of veno-occlusive disease in PSCT recipients with no evidence of reversal; (iii) the patient had moderate or severe liver disease, as defined by aspartate transaminase (AST) or alanine aminotransferase (ALT) levels >10 times the upper limit of normal (ULN), total bilirubin levels >5 times the ULN, or serum alkaline phosphatase levels >10 times the ULN; (iv) the patient had serum creatinine levels >2 times the ULN; (v) the patient had hypokalemia of <3.0 meq/liter; (vi) the patient had a history of anaphylaxis attributed to amphotericin B; and (vii) the patient had received other systemically administered antifungal agents.

The protocol was designed as an open-label, sequential-dose-escalation, multidose pharmacokinetic study that enrolled eight patients in each of the four dosage cohorts. A dosage of 2.5 mg/kg, 5.0 mg/kg, 7.5 mg/kg, or 10 mg/kg of L-AMB was administered once daily as a 1-h infusion to eight patients in each dosage cohort. Escalation and enrollment into the next dosage cohort were permitted only after evaluation of the safety and tolerability for the patient group receiving the lower dosage. Administration was continued for a period of at least 3 days and was discontinued upon recovery from neutropenia (neutrophil counts of >250 neutrophils/$\mu$l). Patients were permitted to reenroll in the trial only after a minimum washout period of 1 month.

**Monitoring of safety and tolerability.** The following laboratory examinations were performed on days 3, 5, and 7 and twice weekly while on the study drug and on the last day of dosing: hemoglobin, hematocrit, total white blood cell count with differential, platelet count, blood urea nitrogen (BUN), serum creatinine, calcium, potassium, sodium, AST, ALT, alkaline phosphatase, total bilirubin, magnesium, phosphorus, glucose, and complete urinalysis. Lipase, amylase, and cholesterol levels were measured on day 7 weekly and on the last day of study drug administration. Determination of the serum creatinine level was performed by using the Jaffe method of analysis (46).

Infusion-related toxicity was monitored prospectively for each infusion of the study drug. Patients were not premedicated with acetaminophen, diphenhydramine, meperidine, or hydrocortisone for the administration of the first dose of L-AMB, thus permitting an evaluation of infusion-related toxicity. If infusion-related symptoms developed during the first infusion, one or more of the above-mentioned agents could be administered. A bedside data extraction sheet was utilized by the nursing staff to record serial vital signs during and after infusion as well as signs and symptoms of infusion-related toxicity. This data extraction sheet then became a source document for reporting infusion-related toxicity. Vital signs were monitored immediately before infusion; 5, 10, 15, and 30 min into the infusion; at the end of the infusion; and 30 and 60 min postinfusion on the first day. Upon subsequent infusions, vital signs were obtained preinfusion, 15 and 30 min into the infusion, at the end of infusion, and 30 and 60 min postinfusion. Between doses, vital signs were obtained every 4 h. Signs, symptoms, and reported side effects associated with study drug infusion or occurring at any time during the study period were recorded and assessed for a relationship to the study drug. The relationship of the study drug to possible clinical infusion-related toxicity was assessed by each patient's primary physician.

Data for safety and tolerability were carefully assessed before escalation to the next dosage cohort. Six of eight patients were required to complete therapy with no significant drug-related grade 3 or grade 4 toxicity according to NCI common toxicity criteria (https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf). Escalation to the next-higher-dosage level was permitted after mutual agreement between the investigator and the clinical monitor that safety criteria had been fulfilled.

**Pharmacokinetic sampling.** Two-milliliter venous blood samples were centrifuged, and the serum fraction was stored at −70°C until analysis. First-dose pharmacokinetic sample collection times were as follows: prior to dosing; at 1 h (end of infusion); and at 1.5, 2.0, 3, 4, 6, 8, 12, 18, and 24 h. Twice-a-week trough samples were subsequently obtained immediately prior to the next dose during daily administration. Last-dose pharmacokinetic samples were then obtained prior to dosing; at 1 h (end of infusion); and at 1.5, 2.0, 3, 4, 6, 8, 12, 18, and 24 h, followed by the collection of washout samples 2, 3, and 4 days (±24 h) after the last dose of L-AMB.

**Analytical methods.** Concentrations of amphotericin B in serum were determined by a high-performance liquid chromatography (HPLC) assay (3). Following methanol deproteinization, amphotericin B and the internal standard 3-nitrophenol were separated by reversed-phase chromatography and detected by UV absorbance at 406 nm. Two overlapping standard curves were used: 0.05 to 20 $\mu$g/ml and 0.5 to 200 $\mu$g/ml. The unweighted correlation coefficient for this assay was 0.998 for both curves, with interday and intraday coefficients of variation (CV) of 1.8 to 11.2% and 6.9 to 10.1%, respectively.

**Pharmacokinetic calculations.** The pharmacokinetic profile of amphotericin B following L-AMB administration was determined by noncompartmental analysis. $C_{max}$ was determined as the maximum concentration of L-AMB measured. The terminal elimination half-life ($t_{1/2}$) was obtained from data in the postdistribution phase. The elimination rate constant $\beta$ was defined as $0.693/t_{1/2}$. The $AUC_{0-24}$ was calculated by using the linear trapezoidal method. $AUC_{inf}$ was determined as $AUC_{24} + AUC_{t-inf}$, with $AUC_{t-inf}$ being extrapolated from $C_t\beta$, where $C_t$ is the last measured concentration. Total body clearance (CL) was calculated as dose/$AUC_{inf}$. The volume of distribution ($V$) was calculated as $V = CL/\beta$.

**Monitoring of efficacy.** This study was not designed for assessments of efficacy. Nonetheless, patients with documented infections known at baseline were evaluated for the response to antifungal therapy as a secondary objective of the clinical trial. Serial blood cultures, urine cultures, and chest radiographs were performed on all febrile neutropenic patients as appropriate. Computerized tomo-

graphic scans and bronchoalveolar lavage were performed as appropriate for evaluating patients for suspected invasive fungal infections. The response of patients with documented baseline infection was assessed by the investigator using clinical, radiological, and microbiological criteria for a complete response, partial response, stabilization, or failure. For patients enrolled for empirical therapy, success was defined as a complete response (resolution of fever and clinical signs and symptoms) or a partial response (improvement but not complete resolution of clinical signs and symptoms); failure of empirical therapy was defined as death, breakthrough fungal infection, or withdrawal due to an adverse event.

**Statistical analysis.** Comparisons of the mean pharmacokinetic values between different dosage levels of L-AMB were performed by analysis of variance (ANOVA) with Dunnett's correction for multiple comparisons. Differences in mean clinical laboratory values and indicators of tolerability of the study drug were analyzed by a Wilcoxon rank sum test. Dichotomous variables of adverse events and infusion-related reactions were analyzed by Fisher's exact test. A $P$ value of $\leq 0.05$ was considered to indicate a significant difference.

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