Liposomal Amphotericin B as Initial Therapy for Invasive Mold Infection: A Randomized Trial Comparing a High–Loading Dose Regimen with Standard Dosing (AmBiLoad Trial)

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(See the editorial commentary by Anaissie on pages 1298–1306)

Background. Treatment of invasive mold infection in immunocompromised patients remains challenging. Voriconazole has been shown to have efficacy and survival benefits over amphotericin B deoxycholate, but its utility is limited by drug interactions. Liposomal amphotericin B achieves maximum plasma levels at a dosage of 10 mg/kg per day, but clinical efficacy data for higher doses are lacking.

Methods. In a double-blind trial, patients with proven or probable invasive mold infection were randomized to receive liposomal amphotericin B at either 3 or 10 mg/kg per day for 14 days, followed by 3 mg/kg per day. The primary end point was favorable (i.e., complete or partial) response at the end of study drug treatment. Survival and safety outcomes were also evaluated.

Results. Of 201 patients with confirmed invasive mold infection, 107 received the 3-mg/kg daily dose, and 94 received the 10-mg/kg daily dose. Invasive aspergillosis accounted for 97% of cases. Hematological malignancies were present in 93% of patients, and 73% of patients were neutropenic at baseline. A favorable response was achieved in 50% and 46% of patients in the 3- and 10-mg/kg groups, respectively (difference, 4%; 95% confidence interval, 1% to 10%; \( P > .05 \)); the respective survival rates at 12 weeks were 72% and 59% (difference, 13%; 95% confidence interval, 2% to 26%; \( P > .05 \)). Significantly higher rates of nephrotoxicity and hypokalemia were seen in the high-dose group.

Conclusions. In highly immunocompromised patients, the effectiveness of 3 mg/kg of liposomal amphotericin B per day as first-line therapy for invasive aspergillosis is demonstrated, with a response rate of 50% and a 12-week survival rate of 72%. The regimen of 10 mg/kg per day demonstrated no additional benefit and higher rates of nephrotoxicity.

Invasive mold infections continue to account for significant morbidity and mortality in immunocompromised patients. Recent epidemiological studies indicate that the incidence of these infections is increasing, although specific risks vary for different underlying diseases [1–3]. Voriconazole has demonstrated efficacy and survival benefits over amphotericin B deoxycholate for the treatment of invasive aspergillosis, but it has numerous drug interactions and no activity against Zygomycetes, limiting its utility for this patient population [4]. Caspofungin has documented efficacy as salvage therapy for patients with invasive aspergillosis, but data are lacking for use as primary therapy [5]. Amphotericin B deoxycholate is fungicidal against Aspergillus species, Zygomycetes, and other molds. However, it has poor efficacy in immunosuppressed patients and is

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associated with significant toxicities [6]. Liposomal amphotericin B has been shown to have a better safety profile than both the deoxycholate and lipid complex forms of amphotericin B, and it can be given at higher doses [7–9].

Liposomal amphotericin B pharmacokinetics are nonlinear, with maximum plasma concentrations and areas under the curve achieved at a dosage of 10 mg/kg per day. The maximum tolerated dosage exceeds 15 mg/kg per day, and the safety profile for dosages of 7.5–15 mg/kg per day has been documented [9]. The licensed dosage for treatment of confirmed invasive fungal infections is 3–5 mg/kg per day. To test the hypothesis that administration of higher doses of liposomal amphotericin B could improve outcome, as suggested by animal study data [10], we performed a prospective, randomized, double-blind trial comparing a high-dose regimen (10 mg/kg per day) with a standard-dose regimen (3 mg/kg per day) of liposomal amphotericin B (AmBisome; Gilead Sciences) for initial treatment of invasive aspergillosis and other mold infections.

METHODS

The study was conducted at 71 sites in 10 countries in Europe and Australia. The protocol was approved by the appropriate national and/or local ethics committees for each participating site. Written informed consent was obtained for each patient who enrolled in the trial. An independent data safety committee monitored the trial. Eligible patients met criteria for proven or probable invasive mold infections established by the European Organization for Research and Treatment of Cancer (EORTC)/Mycosis Study Group (MSG) [11]. As in a recently published study methodology [6], a protocol-defined modification to these criteria allowed a diagnosis of probable invasive aspergillosis in patients with a halo or air crescent sign on chest CT who had undergone allogeneic stem cell transplantation or who had neutropenia (absolute neutrophil count, <500 cells/mm³) within 14 days of study entry. Patients were ineligible if they had received ≥4 days of systemic antifungal therapy for the currently diagnosed fungal infection; had received ≥4 days of systemic polyene therapy within 2 weeks before study entry; had a serum creatinine level ≥2 times the upper limit of normal; had aminotransferase, bilirubin, or alkaline phosphatase levels >5 times the upper limit of normal; had a history of hypersensitivity to amphotericin B products; or had a life expectancy of <30 days.

Patients with a diagnosis of possible invasive mold infection, as determined using the EORTC/MSG criteria (i.e., a host factor plus either a clinical or microbiological criterion), were allowed to enroll in the study and to begin treatment with the randomized study drug. These patients were required to have a proven or probable diagnosis established within 4 working days of enrollment to continue study drug treatment. Patients who did not fulfill the criteria for proven or probable infection within this time frame were disqualified from the analysis populations.

Patients who participated in the trial were randomized to receive a 14-day course of blinded study drug treatment, which consisted of liposomal amphotericin B at 3 mg/kg per day (the standard-dose group) or 10 mg/kg per day (the high-dose group). After day 14 of treatment, all patients received the open-label drug at a dosage of 3 mg/kg per day, until an investigator determined the end of study drug treatment. Study drug treatment could end if there was an efficacy response (favorable or unfavorable), an adverse event that prompted a change in antifungal therapy, death, or other reasons for patients who discontinued treatment within the study protocol.

The study drug was prepared by an unblinded pharmacist. Investigators and site personnel involved in direct care of the patients remained blinded throughout the trial with regard to the dose administered during the first 14 days of treatment. Cover bags and opaque intravenous tubing were used to maintain blinded conditions. Volumes of drug infusions were identical for both dose regimens and were based on patient weight, maintaining the recommended drug concentration for infusion of 0.2–2.0 mg/mL, in accordance with the package insert for liposomal amphotericin B [12]. All infusions were given over a minimum of 2 h.

Serum galactomannan assays (Platelia; Bio-Rad) were performed at each site or at a central laboratory (Covance Laboratory; Geneva, Switzerland) for sites that lacked this test. An optical density index of ≥1.0 was considered to indicate a positive result.

The overall response assessment was based on clinical, radiological, and (if available) microbiological findings at the end of the study drug treatment regimen. Response criteria used have been described elsewhere [6]. Favorable overall responses consisted of complete and partial responses. Stable responses, failures, and unfavorable cases were counted as unfavorable overall responses. Survival was monitored for 12 weeks after study entry. Death was considered a failure unless autopsy findings were available and confirmed no pathological and microbiological evidence of invasive fungal infection.

The intent-to-treat population consisted of all randomized patients who received at least 1 dose of study drug and who had a protocol-defined diagnosis of proven or probable invasive mold infection by the fourth working day of study treatment. An independent data review board, which consisted of 3 clinicians and 1 radiologist blinded to study drug dose received, evaluated all cases in the intent-to-treat population to verify diagnoses and overall response assessments. All adjudications were reached by consensus. Patients with data review board–confirmed cases of invasive mold infection constituted the modified intent-to-treat population. This population was used...
for the primary response and survival analyses. The safety analysis was performed on the intent-to-treat population.

The primary objective of the trial was to compare overall response at the end of the study treatment regimen for high-dose (10 mg/kg per day for 14 days, followed by 3 mg/kg per day) with standard dose (3 mg/kg per day) liposomal amphotericin B in the modified intent-to-treat population. Secondary end points included survival up to 12 weeks and the safety profiles of the treatment regimens.

Patients were centrally randomized in a 1:1 ratio using an automated Interactive Voice Response System. Randomization was stratified for the following factors to maintain balance between treatment groups: (1) age (<18 vs. ≥18 years), (2) type of infection (pulmonary vs. extrapulmonary), (3) allogeneic stem cell transplantation (yes vs. no), and (4) duration of neutropenia at baseline (<14 vs. ≥14 days).

The primary end point was analyzed by Cochran-Mantel-Haenszel procedure for general association (adjusting for strata) on the 2×2 table (2 treatments and 2 outcomes). The analysis adjusted for duration of neutropenia and transplantation status at baseline. Kaplan-Meier procedure was used to estimate the time to event end points. Ninety-five percent CIs for differences in treatments were used for secondary end points. Fisher’s exact test was used to compare safety data for the 2 treatment groups. A sample size of 200 patients (100 subjects per group) was determined to have 82% power to detect the difference between the standard-dose group (with 50% response rate) and the high-dose group (with 70% response rate; OR, 2.333) at a .05 significance level.

RESULTS

Enrollment and baseline characteristics. A total of 339 patients were enrolled during the period from April 2003 through October 2004. Patient disposition is shown in figure 1. Eight patients enrolled in the study but never received study drug. Of the remaining 331 patients, 105 (32%) did not have a proven or probable diagnosis of invasive fungal infection established within 4 working days after enrollment and were disqualified from the efficacy analysis. The data review board verified infections in 201 (89%) of the 226 patients with investigator-determined invasive fungal infections; 107 patients were in the standard-dose group, and 94 were in the high-dose group. Of the patients rejected by the data review board, most were rejected because of an absence of halo or air crescent signs on the chest CT and because the patients had no microbiologic evidence of fungal infection (7 patients in the standard-dose group and 15 in the high-dose group). Other reasons for rejection included the isolation of Candida species rather than mold species (1 patient in each group) and the lack of host factor criteria (1 patient in the high-dose group).

The demographic characteristics and underlying conditions for patients in the modified intent-to-treat population are summarized in table 1. Baseline characteristics were similar for the 2 treatment groups. Hematologic malignancies were present in 93% of patients in each treatment group. The majority of subjects had evidence of uncontrolled disease (i.e., active malignancy) at the time of study entry.

Characteristics of the fungal infections and diagnostic criteria are summarized in table 1. For patients who received a diagnosis of probable invasive pulmonary aspergillosis on the basis of allogeneic stem cell transplantation or neutropenia and chest CT findings only, all had ≥1 halo sign present, which is indicative of early, active infection.

Treatment. Among the patients with investigator-determined invasive fungal infection, 66% in the standard-dose group and 50% in the high-dose group completed 14 days of randomized study drug treatment. The primary factor accounting for this difference was discontinuation of treatment because of adverse events (15 [13%] of 115 patients in the standard-dose group vs. 27 [24%] of 111 in the high-dose group; \( P = .04 \)). The median duration of study drug treatment in the modified intent-to-treat population was 15 days (range, 1–60 days) in the standard-dose group and 14 days (range, 1–57 days) in the high-dose group.

After study drug treatment was discontinued, additional antifungal therapy was administered to 70% of standard-dose treatment recipients and 68% of high-dose treatment recipients. The most commonly used agents were voriconazole (intravenous or oral; 53% and 54% of patients in the standard- and high-dose groups, respectively) and caspofungin (20% and 17%, respectively).
Table 1. Characteristics of patients in a study of liposomal amphotericin B as initial therapy for invasive mold infection.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Liposomal amphotericin B dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 mg/kg per day (n = 107)</td>
</tr>
<tr>
<td></td>
<td>10 mg/kg per day (n = 94)</td>
</tr>
<tr>
<td></td>
<td>(n)</td>
</tr>
<tr>
<td>Baseline characteristic</td>
<td></td>
</tr>
<tr>
<td>Age, mean years (range)</td>
<td>50.9 (15–76)</td>
</tr>
<tr>
<td></td>
<td>50.4 (2–78)</td>
</tr>
<tr>
<td>Age &lt;18 years, no. of patients</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Percentage of male/female patients</td>
<td>57/43</td>
</tr>
<tr>
<td></td>
<td>67/33</td>
</tr>
<tr>
<td>Weight, mean kg</td>
<td>68.3</td>
</tr>
<tr>
<td></td>
<td>71.0</td>
</tr>
<tr>
<td>Underlying condition</td>
<td></td>
</tr>
<tr>
<td>Hematological malignancies&lt;sup&gt;a&lt;/sup&gt;</td>
<td>99 (93)</td>
</tr>
<tr>
<td></td>
<td>87 (93)</td>
</tr>
<tr>
<td>Controlled</td>
<td>36/99 (36)</td>
</tr>
<tr>
<td></td>
<td>26/85 (31)&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>Uncontrolled</td>
<td>63/99 (64)</td>
</tr>
<tr>
<td></td>
<td>59/85 (69)&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stem cell transplantation</td>
<td></td>
</tr>
<tr>
<td>Allogeneic</td>
<td>17 (16)</td>
</tr>
<tr>
<td></td>
<td>18 (19)</td>
</tr>
<tr>
<td>Autologous</td>
<td>1 (1)</td>
</tr>
<tr>
<td></td>
<td>4 (4)</td>
</tr>
<tr>
<td>Solid organ transplantation</td>
<td>1 (1)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>HIV infection</td>
<td>2 (2)</td>
</tr>
<tr>
<td></td>
<td>2 (2)</td>
</tr>
<tr>
<td>Other&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5 (5)</td>
</tr>
<tr>
<td></td>
<td>2 (2)</td>
</tr>
<tr>
<td>Neutropenia&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Within 60 days of enrollment</td>
<td>97 (91)</td>
</tr>
<tr>
<td></td>
<td>84 (89)</td>
</tr>
<tr>
<td>Neutropenia at baseline</td>
<td>76 (71)</td>
</tr>
<tr>
<td></td>
<td>71 (76)</td>
</tr>
<tr>
<td>Site of infection</td>
<td></td>
</tr>
<tr>
<td>Pulmonary</td>
<td>98 (92)</td>
</tr>
<tr>
<td></td>
<td>84 (89)</td>
</tr>
<tr>
<td>Disseminated</td>
<td>3 (3)</td>
</tr>
<tr>
<td></td>
<td>4 (4)</td>
</tr>
<tr>
<td>Other localized site&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6 (6)</td>
</tr>
<tr>
<td></td>
<td>6 (6)</td>
</tr>
<tr>
<td>Fungal pathogen</td>
<td></td>
</tr>
<tr>
<td>Proven or probable Aspergillus&lt;sup&gt;f&lt;/sup&gt;</td>
<td>103 (96)</td>
</tr>
<tr>
<td></td>
<td>91 (97)</td>
</tr>
<tr>
<td>Aspergillus plus Alternaria species</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1 (1)</td>
</tr>
<tr>
<td>Zygomycetes</td>
<td>3 (3)</td>
</tr>
<tr>
<td></td>
<td>2 (2)</td>
</tr>
<tr>
<td>Phaeoacremonium species</td>
<td>1 (1)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Invasive aspergilosis</td>
<td></td>
</tr>
<tr>
<td>Microbiologically confirmed</td>
<td>41 (38)</td>
</tr>
<tr>
<td></td>
<td>36 (38)</td>
</tr>
<tr>
<td>Halo sign on CT only&lt;sup&gt;f,g&lt;/sup&gt;</td>
<td>62 (58)</td>
</tr>
<tr>
<td></td>
<td>56 (60)</td>
</tr>
<tr>
<td>Microbiologic criteria</td>
<td></td>
</tr>
<tr>
<td>Aspergillus antigen only&lt;sup&gt;h&lt;/sup&gt;</td>
<td>27 (25)</td>
</tr>
<tr>
<td></td>
<td>18 (19)</td>
</tr>
<tr>
<td>Culture and/or histologic findings only</td>
<td>14 (13)</td>
</tr>
<tr>
<td></td>
<td>16 (17)</td>
</tr>
<tr>
<td>Aspergillus antigen&lt;sup&gt;h&lt;/sup&gt; plus culture and/or histologic findings</td>
<td>4 (4)</td>
</tr>
<tr>
<td></td>
<td>4 (4)</td>
</tr>
<tr>
<td>Data Review Board–confirmed case</td>
<td></td>
</tr>
<tr>
<td>Proven</td>
<td>8 (7)</td>
</tr>
<tr>
<td></td>
<td>10 (11)</td>
</tr>
<tr>
<td>Probable</td>
<td>99 (93)</td>
</tr>
<tr>
<td></td>
<td>84 (89)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of patients, unless otherwise indicated.

<sup>a</sup> Includes acute and chronic leukemia, lymphoma, multiple myeloma, and myelodysplastic syndrome. “Controlled” was defined as no active malignant disease present at baseline (i.e., complete remission), and “uncontrolled” was defined as active malignant disease present at baseline.

<sup>b</sup> Data were missing for 2 patients.

<sup>c</sup> Conditions requiring chronic corticosteroid therapy.

<sup>d</sup> Absolute neutrophil count, <500 cells/mm<sup>3</sup>.

<sup>e</sup> Sites include CNS, sinus, nasal, neck, sternal wound, hepatosplenic sites, kidney, scrotum, skin, knee, and leg.

<sup>f</sup> Protocol modification of European Organization for Research and Treatment of Cancer criteria: CT halo or air crescent sign in allogeneic stem cell transplant recipients or recent neutropenia were categorized as probable aspergillosis.

<sup>g</sup> All 118 patients had halo signs (single or multiple) on baseline chest CTs, 4 patients had an air crescent sign in addition to halo signs, and 1 patient had multiple halos plus a cavitary lesion.

<sup>h</sup> Two serum samples or 1 bronchoalveolar lavage sample tested positive.
Outcomes. Favorable overall response results at the end of study drug treatment are summarized in table 2. There was no statistically significant difference with regard to favorable overall responses between the treatment groups (50% in the standard-dose group vs. 46% in the high-dose group; \( P = .65 \)). Invasive aspergillosis was identified in 103 patients in the standard-dose group and 92 patients in the high-dose group. Favorable overall responses for these subgroups were 50% and 46%, respectively, as well. The subset of patients with probable invasive aspergillosis based solely on the presence of halo signs on chest CT had rates of favorable response of 56% (standard-dose group) and 48% (high-dose group).

The rate of survival at the end of study drug treatment was 93% in the standard-dose group and 88% in the high-dose group (difference, 4%; 95% CI, \(-4\% \) to 12%; \( P > .05 \)). At 12 weeks after study entry, the survival rates were 72% and 59% for the standard- and high-dose groups, respectively (difference, 13%; 95% CI, \(-0.2\% \) to 26%; \( P > .05 \)). The stratified \( p \) value for the log rank test of Kaplan-Meier estimates of time to death during the course of the study was .089 (figure 2). The rates of survival at 12 weeks for the invasive aspergillosis subsets were 71% and 58%.

Differences in the Kaplan-Meier curves for the 2 treatment groups do not become evident until well after the initial 2-week randomized treatment period. This suggests that factors other than initial treatment dose received may have impacted survival. A multivariate stepwise logistic regression analysis was performed to identify factors associated with survival at 12 weeks. Significant negative associations for survival were found for the baseline factors of allogeneic stem cell transplantation (the 12-week survival rate was 40%, compared with 71% for no transplantation; difference, 31%; 95% CI, 14%–49%) and uncontrolled hematological malignancy (12-week survival, 54% vs. 81% for controlled malignancy; difference, 27%; 95% CI, 15%–39%). Randomized treatment group was not associated with a survival difference by this analysis.

Subset analyses revealed no significant differences in favorable overall response and 12-week survival rates between treatment groups on the basis of site of infection, underlying condition (including the allogeneic stem cell transplantation and uncontrolled hematological malignancy subgroups), and neutropenia status at baseline. Similar results for overall response and survival rates were found in the intent-to-treat population (data not shown).

Safety. Adverse event and laboratory toxicity data for the intent-to-treat population are presented in table 3. Nephrotoxicity, defined as an increase in the serum creatinine level to twice the baseline value, occurred at a greater rate in the high-dose group (31% vs. 14%; \( P < .01 \)). Grade 3 hypokalemia (blood potassium level, <3.0 mmol/L) was also more frequently found in the high-dose group (30% vs. 16%; \( P = .015 \)). There was no difference between the groups with regard to the rates of grade 4 hypokalemia (blood potassium level, <2.5 mmol/L).

No differences in the rates of drug-related reactions, including

Table 2. Favorable overall responses among all patients and subsets of patients.

<table>
<thead>
<tr>
<th>Patient group or characteristic</th>
<th>Percentage of patients with favorable overall response, by liposomal amphotericin B dosage</th>
<th>Difference, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 mg/kg per day</td>
<td>10 mg/kg per day</td>
</tr>
<tr>
<td>All patientsa</td>
<td>50</td>
<td>46</td>
</tr>
<tr>
<td>All patients with aspergillosis</td>
<td>50</td>
<td>46</td>
</tr>
<tr>
<td>Patients with microbiologically confirmed aspergillosis</td>
<td>39</td>
<td>42</td>
</tr>
<tr>
<td>Patients with aspergillosis diagnosed by presence of halo sign only</td>
<td>56</td>
<td>48</td>
</tr>
<tr>
<td>Allogeneic stem cell transplantation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>47</td>
<td>50</td>
</tr>
<tr>
<td>No</td>
<td>50</td>
<td>45</td>
</tr>
<tr>
<td>Hematological malignancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controlledb</td>
<td>53</td>
<td>54</td>
</tr>
<tr>
<td>Uncontrolledb</td>
<td>48</td>
<td>44</td>
</tr>
<tr>
<td>Neutropenia at baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>43</td>
<td>42</td>
</tr>
<tr>
<td>No</td>
<td>67</td>
<td>57</td>
</tr>
<tr>
<td>Pulmonary infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extrapulmonary infection</td>
<td>51</td>
<td>48</td>
</tr>
</tbody>
</table>

\( ^a \) Categorical responses: favorable overall response for 3 mg/kg and 10 mg/kg treatment arms is the sum of complete response (1% and 2%, respectively) and partial response (49% and 44%, respectively). Unfavorable response includes stable disease (7% and 5%, respectively), treatment failure (34% and 38%, respectively), and cases that were not evaluable (9% and 11%, respectively).

\( ^b \) Controlled was defined as complete remission, and uncontrolled was defined as active malignancy present at baseline.
hypersensitivity/anaphylaxis, chills, or hypotension, were reported. No unusual or previously unrecognized safety signals were seen in either treatment arm. Similar findings were seen in the “all-treated” population, which included patients who had been disqualified from study drug treatment after working day 4.

There was a difference in the rates of study drug discontinuation resulting from adverse events (20% in the standard-dose group and 32% in the high-dose group; \( p = .035 \)). The most common events leading to study drug discontinuations in both groups were increases in the creatinine level, abnormal liver test results, and hypokalemia.

**DISCUSSION**

This study represents the first large-scale, randomized, prospective efficacy trial of liposomal amphotericin B to use strictly defined criteria for proven and probable invasive mold infections. The data demonstrate similar response rates for a 3-mg/kg daily regimen, compared with a high-dose regimen of 10 mg/kg per day for the first 14 days of treatment, for both overall response and survival.

Patients were recruited when they had the first signs or symptoms of fungal disease, and confirmation of the diagnosis was mandatory within 4 working days after study entry. This approach was used because there are often delays in obtaining microbiological results to confirm invasive fungal infections and because of the need to initiate antifungal therapy as soon as possible, to treat these infections most effectively.

Previously published studies have reported efficacy of liposomal amphotericin B in invasive mold infection [9, 13–18]. Because these studies were performed before the most recent standardization of the diagnostic criteria for invasive fungal infection, a number of cases included would not qualify as proven or probable infection on the basis of the current definitions [11]. A pooled retrospective analysis has recently been performed, using current diagnostic standards to screen patients from these studies [19]. Of 212 cases reviewed, 69 had clinical, radiological, and microbiological evidence of invasive mold infection. The median treatment dosage for these infections was 4 mg/kg per day (range, 1–15 mg/kg per day). Favorable response and survival rates were both 51%. In 44 patients who received liposomal amphotericin B as first-line therapy, the response rate was 61%.

The 50% response rate in the standard-dose group and the 12-week survival rate of 72% in the present study are comparable to rates reported for voriconazole and exceed rates for amphotericin B deoxycholate in a recent trial [6]. This trial is similar to the study by Herbrecht et al. [6] in terms of the criteria used for diagnosis of invasive fungal infection and response assessments, as well as the use of independent data review committees blinded to the study drug assignment who verified fungal infection diagnoses and response assessments.
Table 3. Most commonly reported adverse events related to receipt of the study drug and laboratory abnormalities.

<table>
<thead>
<tr>
<th>Event</th>
<th>3 mg/kg per day (n = 115)</th>
<th>10 mg/kg per day (n = 111)</th>
<th>Difference, % (95% CI)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypokalemia ab</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium level, &lt;3.0 mmol/L</td>
<td>18 (16)</td>
<td>32 (30)</td>
<td>-14 (-25 to -3)</td>
<td>.015</td>
</tr>
<tr>
<td>Potassium level, &lt;2.5 mmol/L</td>
<td>3 (3)</td>
<td>4 (4)</td>
<td>-1 (-6 to 4)</td>
<td></td>
</tr>
<tr>
<td>Increase in creatinine levelc</td>
<td>12 (10)</td>
<td>30 (27)</td>
<td>-17 (-27 to -7)</td>
<td>.002</td>
</tr>
<tr>
<td>Doubling of creatinine levelc</td>
<td>16 (14)</td>
<td>31 (31)</td>
<td>-17 (-28 to -5)</td>
<td>.005</td>
</tr>
<tr>
<td>Abnormal liver function test resultsb, e</td>
<td>18 (16)</td>
<td>16 (14)</td>
<td>-1 (-8 to 11)</td>
<td></td>
</tr>
<tr>
<td>Increased bilirubin levelf</td>
<td>9 (8)</td>
<td>11 (10)</td>
<td>-2 (-9 to 5)</td>
<td></td>
</tr>
<tr>
<td>Increased alkaline phosphatase levelc</td>
<td>7 (6)</td>
<td>10 (9)</td>
<td>-3 (-10 to 4)</td>
<td></td>
</tr>
<tr>
<td>Increased alanine aminotransferase levelc</td>
<td>5 (4)</td>
<td>9 (8)</td>
<td>-4 (-10 to 3)</td>
<td></td>
</tr>
<tr>
<td>Increased aspartate aminotransferase levelc</td>
<td>1 (&lt;1)</td>
<td>5 (5)</td>
<td>-4 (-8 to 1)</td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>11 (10)</td>
<td>14 (13)</td>
<td>-3 (-11 to 5)</td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>5 (4)</td>
<td>5 (5)</td>
<td>0 (-6 to 5)</td>
<td></td>
</tr>
<tr>
<td>Chills</td>
<td>4 (3)</td>
<td>9 (8)</td>
<td>-5 (-11 to 1)</td>
<td></td>
</tr>
<tr>
<td>Hypersensitivity</td>
<td>1 (&lt;1)</td>
<td>5 (5)</td>
<td>-3 (-7 to 1)</td>
<td></td>
</tr>
<tr>
<td>Dyspnea</td>
<td>3 (3)</td>
<td>4 (4)</td>
<td>-1 (-6 to 4)</td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>5 (4)</td>
<td>1 (&lt;1)</td>
<td>3 (-1 to 8)</td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1 (&lt;1)</td>
<td>4 (4)</td>
<td>-3 (-7 to 1)</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Treatment emergent events (i.e., those that were not present at baseline) occurred up to the 4-week post-end-of-therapy follow-up (incidence in either treatment group, ≥4%).

* Only significant values are reported.

a Hypokalemia with serum potassium level <3.0 mmol/L.

b Treatment-emergent laboratory abnormalities.

c Investigator-reported adverse events.

d Serum creatinine level ≥2 times the baseline level.

e Includes all patients with the treatment-emergent laboratory abnormalities of increases in the alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase (>5 times the upper limit of normal), or total bilirubin (>3 times the upper limit of normal) level.

In both trials, the presence of halo and air crescent signs on chest CTs for patients who had undergone allogeneic stem cell transplantation or who had recent neutropenia was used to define probable aspergillosis, even in the absence of microbiologic confirmation. This approach was recently supported by an expert panel [20]. The patient population in the present study differed from the population in the voriconazole trial with respect to risk factors. Our study included a greater proportion of neutropenic patients (73% vs. 45%). In addition, the vast majority of patients in our study had uncontrolled (i.e., active) underlying hematological malignancies at the time of enrollment.

This trial included cases of invasive mold infection other than aspergillosis (5 cases due to Zygomycetes and 1 case due to Phaeoacremonium species). Treatment was successful for one-half of these patients, reflecting the broad spectrum of liposomal amphotericin B. Liposomal amphotericin B offers an advantage over echinocandins and most azoles, because in clinical practice, the etiologic agent in invasive fungal infections often remains unidentified.

It is worth noting that the primary response assessment occurred at the end of study drug treatment (median duration, 14–15 days). In patients with invasive pulmonary aspergillosis, chest CT abnormalities (including nodular lesions with halos, cavitary lesions, and air space consolidation) are frequently known to increase in size over this time frame [21]. Patients assessed at this time point in our trial who had worsening chest CT findings were judged to have experienced treatment failure, although subsequent clinical and radiographic improvement may have occurred with further treatment. Thus, the long-term response rates may be underestimated because of this aspect of the study design. For similar reasons, most favorable assessments were partial responses, because many patients were evaluated at a time point when complete radiological clearing of lesions would not be expected to occur.

The safety profiles for both dose regimens in this study are comparable to previously reported data. More than 1300 patients received liposomal amphotericin B at 3 mg/kg per day in 3 major clinical trials of empirical antifungal therapy for febrile neutropenia [7, 22, 23]. In this study, rates of nephrotoxicity and hypokalemia were similar to rates reported in the 3 empirical therapy trials, suggesting that there are no addi-
tional safety concerns for patients with documented invasive mold infection. Increasing the dosage to 10 mg/kg per day results in higher rates of nephrotoxicity and hypokalemia, as has been suggested elsewhere [9].

Although no differences between the treatment arms in outcomes were noted in this study, it is important to recognize the impact of the underlying patient factors of allogeneic stem cell transplantation and presence of active hematological malignancy at baseline on overall survival. Significantly lower rates of survival at week 12, regardless of treatment regimen received, were associated with each of these factors. This has prognostic implications for patients being treated for invasive mold infection, as well as implications for the design of clinical trials. Patients who are enrolled in randomized antifungal efficacy trials should be stratified on the basis of both allogeneic stem cell transplantation status and underlying hematological malignancy status (controlled vs. uncontrolled), to avoid impacting outcomes with confounding biases.

The results of this study establish the effectiveness of liposomal amphotericin B as initial therapy for invasive mold infection—particularly invasive pulmonary aspergillosis—and support use of the standard dosage of 3 mg/kg per day as primary therapy for these infections. No additional efficacy benefit was obtained with a 10 mg/kg per day dosing regimen. Additional studies are needed to determine whether high-dose liposomal amphotericin B treatment may be more effective for extrapulmonary sites of infection or for infection with non-Aspergillus pathogens, such as Zygomycetes.

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