Aerosolized Liposomal Amphotericin B for the Prevention of Invasive Pulmonary Aspergillosis during Prolonged Neutropenia: A Randomized, Placebo-Controlled Trial

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(See the editorial commentary by Perfect on pages 1409–11)

Background. Invasive pulmonary aspergillosis (IPA) is a significant problem in patients with chemotherapy-induced prolonged neutropenia. Because pulmonary deposition of conidia is the first step in developing IPA, we hypothesized that inhalation of liposomal amphotericin B would prevent IPA.

Methods. We performed a randomized, placebo-controlled trial of patients with hematologic disease with expected neutropenia for >10 days. Patients were randomized to receive liposomal amphotericin B or placebo inhalation twice a week, using an adaptive aerosol delivery system, until neutrophil counts increased to >300 cells/mm3. In subsequent neutropenic episodes, the assigned treatment was restarted. The primary endpoint was the occurrence of IPA according to European Organization for Research and the Treatment of Cancer-Mycoses Study Group definitions. Kaplan-Meier curves were compared with log-rank tests for intent-to-treat and on-treatment populations.

Results. A total of 271 patients were studied during 407 neutropenic episodes. According to the intent-to-treat analysis, 18 of 132 patients in the placebo group developed IPA versus 6 of 139 patients in the liposomal amphotericin B group (odds ratio, 0.26; 95% confidence interval, 0.09–0.72; P = .005). According to the on-treatment analysis, 13 of 97 patients receiving placebo versus 2 of 91 receiving liposomal amphotericin B developed IPA (odds ratio, 0.14; 95% confidence interval, 0.02–0.66; P = .007). Some adverse effects, but none serious, in the liposomal amphotericin B group were reported, most frequently coughing (16 patients vs. 1 patient; P = .002).

Conclusion. In high-risk patients, prophylactic inhalation of liposomal amphotericin B significantly reduced the incidence of IPA.

Invasive fungal infections are an important source of morbidity and mortality in patients receiving treatment for hematologic disease. In particular, patients with prolonged neutropenia and/or severe immunosuppression are at increased risk [1]. Invasive pulmonary aspergillosis (IPA) is the most common mold infection [2]. It remains difficult to reliably diagnose IPA early; thus, several preventive approaches have been investigated. Itraconazole and amphotericin B are both active against Aspergillus, but adverse effects hamper their use [3]. Only recently, data on the prophylactic use of orally administered posaconazole, a new broad-spectrum azole, have become available. Compared with fluconazole posaconazole reduced the incidence of IPA in patients with prolonged neutropenia or graft-versus-host disease [4, 5].

The inhalation of Aspergillus conidia is the first step in the pathogenesis of IPA. Therefore, inhalation of amphotericin B may prevent IPA while avoiding sys-
temic adverse effects [6]. Intravenously, liposomal amphotericin B (Ambisome; Gilead Sciences) is better tolerated than conventional amphotericin B. The liposomal carrier also exhibits a pulmonary surfactant-like function [7]. In contrast, the deoxycholate salt used in conventional amphotericin B acts as a detergent, impairing the surfactant function [8]. In a recent tolerability study, more patients receiving inhaled conventional amphotericin B experienced adverse effects than those receiving amphotericin B lipid complex, another amphotericin B lipid formulation [9]. It remains unclear whether these differences in surfactant function influence the tolerability or efficacy of liposomal amphotericin B administered by inhalation [10].

The only currently published randomized clinical trial found no protective effect against IPA for aerosolized conventional amphotericin B, but low event rates precluded definitive conclusions [11]. We hypothesized that the inhalation of liposomal amphotericin B would prevent IPA.

**MATERIALS AND METHODS**

Eligible study participants were adult patients with hematologic disease hospitalized at Erasmus Medical Center (Rotterdam, The Netherlands). Patients had to start chemotherapy within 7 days after enrollment with an anticipated duration of neutropenia (neutrophil count, <500 cells/mm³) ≥ 10 days. This applied to patients receiving chemotherapy and patients undergoing stem cell transplantation. All patients received prophylactic fluconazole treatment. Patients were ineligible if, at entry, they already had evidence of fungal infection in their lungs or sinuses; in the case of pneumonia; if the patient was unable to use a nebulizer; if expected duration of survival was <3 months; and if the patient had previously demonstrated an intolerance to amphotericin B.

**Study design.** This study was a randomized, double-blind, placebo-controlled trial. Randomization was performed using a computer-generated blocked list and stratified for location site, stem cell transplantation, and use of high-efficiency particulate air filtration. Allocation concealment was performed by employees in the Erasmus Medical Center Department of Pharmacy, who delivered syringes with liposomal amphotericin B or a placebo masked for taste, color, and optical density. Patients were randomized only once. If, after a first treatment phase with liposomal amphotericin B or placebo inhalation therapy, other episodes of neutropenia followed as part of the entire treatment plan, the patient continued the same inhalation therapy as initially assigned. An allogeneic transplantation that followed remission induction chemotherapy was considered a separate treatment entity. Therefore, inhalation therapy was not continued during the allogeneic stem cell transplantation that followed chemotherapy. Patients who had not previously entered the study could be included at the time of allogeneic transplantation. Patients discontinued participation in the study if IPA was diagnosed. The institutional review board approved the protocol, and informed consent was obtained.

**Diagnostic procedures throughout the study.** Per protocol, patients underwent high-resolution CT if chest radiography finding were abnormal or at day 5 of unexplained fever despite treatment with antibiotics, and again 7 days later if fever persisted. Patients underwent bronchoscopically guided bronchoalveolar lavage (BAL) of lung lesions. The BAL specimens were cultured for bacteria, mycobacteria, and fungi, and the galactomannan level in BAL specimens was measured (Platelia Aspergillus ELA; Bio-Rad Laboratories). Serum galactomannan levels were measured twice weekly. In cases in which the results of these diagnostic procedures were inconclusive, biopsy specimens of lung lesions were obtained (if not contraindicated).

Intravenous amphotericin B was the treatment of choice for probable IPA until voriconazole became available (March 2002) for the treatment of IPA in The Netherlands. Per protocol, unexplained neutropenic fever for > 6 days was treated intravenously with amphotericin B. Fluconazole therapy was discontinued whenever amphotericin B or voriconazole was administered.

**Inhalation therapy.** Nebulization of liposomal amphotericin B and placebo was performed with an adaptive aerosol delivery system (Halolite AAD or ProDose AAD; Romedic/Medic-Aid). This is an advanced nebulizer adapting to individual breathing patterns. It delivers aerosol only during inspiration and generates particles with an average diameter of 1.9 μm. Optimal deposition in the peripheral lung regions is therefore ensured [12]. From the start of the study until December 2003, the Halolite AAD system was used. During the second half of the study, a more user-friendly ProDose AAD system was used. Both systems generate identical aerosol particles and inhaled mass [13].

A total of 2.5 mL of a 5-mg/mL solution of liposomal amphotericin B or placebo was used for inhalation. Nebulization was performed for 30 min per day on 2 consecutive days per week. This weekly regimen was repeated until neutrophil recovery (neutrophil count, > 300 cells/mm³), with a maximum of 12 inhalations per neutropenic episode.

**Definitions** Two diagnostic definitions of IPA were used. For the primary efficacy end point, the European Organization for Research and the Treatment of Cancer–Mycoses Study Group (EORTC-MSG) definition of proven or probable invasive fungal infections were applied [14]. In brief, patients need to have a host factor, a clinicoradiologic criterion (e.g., halo sign), and a mycologic criterion (e.g., galactomannan detection) to be diagnosed with probable IPA. For the secondary end point of efficacy, a modified version of the EORTC-MSG definition of probable IPA was used. This definition which has previously been applied by others, classify patients without a
mycologic criterion but with the typical halo sign or air-crescent sign also as having probable IPA [11, 15, 16].

Outcomes. The primary goal was to compare the incidence of IPA in accordance with EORTC-MSG definitions. For the intent-to-treat (ITT) population, the follow-up period ended 28 days after neutrophil recovery (>300 cells/mm³) from the last course of chemotherapy. For the on-treatment (OT) population, the follow-up ended 28 days after neutrophil recovery from the last neutropenic episode during which weekly inhalations were administered. The predefined secondary objective was the comparison of the incidence of IPA using the modified EORTC-MSG definitions. All end points were classified by 2 blinded investigators (B.J.R. and L.S.). Other end points were overall mortality, IPA-related mortality, and safety. Creatinine levels before the first and immediately after the last inhalation were compared to evaluate renal toxic effects. Overall mortality was registered until 28 days after neutrophil recovery (neutrophil count, >300 cells/mm³). For patients with IPA, the IPA-related mortality was registered until 24 weeks after the last inhalation. Mortality was considered to be IPA related if clinical and radiologic resolution of the Aspergillus infection had not been documented at the time of death.

Statistical analysis. All analyses were performed on the ITT and OT populations. Patients who received at least 1 inhalation of liposomal amphotericin B were included in the ITT analysis. These patients were observed until 28 days after recovery of neutropenia after the last cycle of chemotherapy, irrespective of whether the patient was able to continue the inhalation therapy after the first course of chemotherapy. For the exceptional patient without neutrophil recovery, the follow-up continued until 28 days after the last inhalation therapy. In the OT analysis, neutropenic episodes were only evaluated in patients who received weekly inhalations during these neutropenic episodes (i.e., further observation was censored at the first neutropenic episode at which weekly inhalations were not applied).

Kaplan-Meier curves for the occurrence of IPA were constructed with neutropenic episodes as the time variable. Comparison of these curves was performed with the log-rank test, taking into account the discrete nature of follow-up evaluations (neutropenic episodes). In view of this discrete time axis, results are expressed as ORs (with 95% CIs) instead of conventionally used hazard ratios, which apply to a continuous time axis. Exact calculations were performed with the LogXact program, version 4.1 (Cytel Software). Median serum creatinine levels were compared with the Wilcoxon signed rank test and proportions with Fisher’s exact test. A 2-sided P value <.05 was considered to be statistically significant. We calculated that the sample size needed to show a reduction of IPA from an assumed 7% with placebo to 1% with liposomal amphotericin B inhalation with 80% power (α = .05) was 340 patients.

RESULTS

From November 2000 through February 2006, 271 patients were enrolled and followed up during 407 neutropenic episodes. During 339 episodes, inhalation therapy was administered. A total of 139 patients received liposomal amphotericin B (177 episodes) and 132 received placebo (162 episodes). The 2 groups were balanced for clinical and hematologic characteristics (table 1). Recruitment of the planned total number of patients turned out to be more difficult and slower than expected. After >5 years of recruitment, it was believed impossible to recruit patients for another estimated 18 months. The decision to stop the study was made by the study investigators at a time when all study results were completely blinded. Because no interim analyses had been performed, the validity of the trial results was not affected.

Prophylactic effect of liposomal amphotericin B inhalation. The analysis of the primary end point for the ITT population showed that 6 (4%) of 139 patients treated with liposomal amphotericin B had developed IPA. A total of 18 (14%) of 132 patients treated with placebo developed IPA. This difference was statistically significant (P = .005), with an OR of 0.26 (95% CI, 0.09–0.72) (figure 1). Three of the 6 cases of IPA in the liposomal amphotericin B group occurred during a second or third neutropenic episode, sometime after the patient had elected not to continue receiving liposomal amphotericin B inhalation therapy (43, 53, and 54 days after the last inhalation with liposomal amphotericin B). A fourth patient developed IPA shortly after he entered the study, when he had received a single liposomal amphotericin B inhalation. Until December 2003, 10 cases of IPA were diagnosed in the placebo group and 4 in the liposomal amphotericin B group. From December 2003 until the end of the study, 8 cases were diagnosed in the placebo group and 2 in the liposomal amphotericin B group. Therefore, no difference in efficacy of both aerosol systems (see “Inhalation therapy,” in the Materials and Methods section) was observed.

The OT analysis showed that 15 patients had developed IPA (2 of 90 patients treated with liposomal amphotericin B versus 13 of 97 treated with placebo). This difference was statistically significant (P = .007), with an OR of 0.14 (95% CI, 0.02–0.66).

The differences between study groups for the secondary efficacy end point of IPA, using the modified EORTC-MSG definition, are displayed in figure 2. For this end point as well, ITT and OT analyses showed a reduction of IPA with liposomal amphotericin B inhalation, with ORs of 0.37 (95% CI, 0.16–0.83) and 0.16 (95% CI, 0.03–0.56), respectively. A total of 11 of 139 patients receiving liposomal amphotericin B developed IPA versus 23 of 132 patients receiving placebo. For the OT population, 3 of 90 patients treated with liposomal amphotericin B developed IPA versus 17 of 97 patients treated with placebo.

Only 1 case of a non-Aspergillus mold infection was diag-
Table 1. Baseline characteristics of the study participants.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Liposomal amphotericin B (n = 139)</th>
<th>Placebo (n = 132)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean years (range)</td>
<td>49 (18–73)</td>
<td>50 (20–74)</td>
<td>.64</td>
</tr>
<tr>
<td>Male sex/female sex</td>
<td>77/62</td>
<td>81/51</td>
<td>.33</td>
</tr>
<tr>
<td>HEPA filtrationa</td>
<td>108</td>
<td>100</td>
<td>.77</td>
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<tr>
<td>Hematologic disease</td>
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<tr>
<td>AML-MDS</td>
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<td>67</td>
<td>.54</td>
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<tr>
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<td>65</td>
<td></td>
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<tr>
<td>Hematologic treatment</td>
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<td>.29</td>
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<tr>
<td>Disease status</td>
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</tr>
<tr>
<td>Untreated</td>
<td>73</td>
<td>64</td>
<td>.54</td>
</tr>
<tr>
<td>Otherb</td>
<td>66</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>Treatment followed by allogeneic HSCTc</td>
<td>16</td>
<td>14</td>
<td>.85</td>
</tr>
</tbody>
</table>

NOTE. Data are no. of patients, unless otherwise indicated. AML, acute myeloid leukemia; HEPA, high-efficiency particulate air; HSCT, hematopoietic stem cell transplantation; MDS, myelodysplastic syndrome.

a Use of HEPA filtration of hospital room air during first course of chemotherapy.
b Partial remission, complete remission, refractory disease, or relapse.
c Inhalation therapy was not continued during allogeneic HSCT that followed intensive chemotherapy.

nosed. This patient, who was treated with placebo, developed a fatal disseminated Fusarium infection. None of the allogeneic stem cell transplant recipients developed IPA.

As required by the EORTC-MSG definition of IPA, all patients with the primary end point had radiologic signs of pulmonary aspergillosis. Two patients also had involvement of another site (brain, 1; cutaneous, 1). The mycologic component of the primary end point definition was galactomannan detection or a culture of Aspergillus species in the BAL specimen in 21 of 24 patients. In 4 cases, galactomannan was found in both BAL fluid and serum specimens. Serum galactomannan was the only mycologic proof of IPA in 3 patients.

Survival and toxic effects. Within the 28 days after neutrophil recovery, 7 patients died in the liposomal amphotericin B group (none of the deaths were IPA related), and 6 patients died in the placebo group (1 death was IPA related). The IPA-related mortality rate was 4.5% (6 of 132 patients) with placebo versus 3.6% (5 of 139 patients) with liposomal amphotericin B for the ITT population (P = .8). For the OT population, the IPA-related mortality rate was 6.2% (6 of 97 patients) with placebo versus 1.1% (1 of 90 patients) with liposomal amphotericin B (P = .1).

In the patients receiving liposomal amphotericin B, median serum creatinine levels after the last inhalation were not greater than the baseline level (59.4 and 64.9 μmol/L, respectively; P = .1). In the placebo group, a trend toward increased creatinine levels was noted (62.7 and 74.6 μmol/L; P = .05).

During the study, significantly more patients in the liposomal amphotericin B group discontinued the inhalation therapy for at least 1 week (45% vs. 30% in the placebo group; P = .01). The most frequently observed reasons for discontinuing treatment were the patient being too weak to use the aerosol delivery system (17 patients in both groups), a technical problem with the aerosol delivery system (12 patients with liposomal amphotericin B, 10 with placebo), and coughing during inhalation. Coughing was observed more frequently with liposomal amphotericin B than with placebo (16 patients vs. 1 patient; P = .002). No drug-related serious adverse events were reported throughout the study.

DISCUSSION

As a result of progressively more dose-intensive myelosuppressive and immunosuppressive therapy for hematologic disease, IPA represents a significant source of morbidity and mortality. In addition, IPA interferes with the delivery of the planned therapy according to schedule. Measures that effectively prevent IPA are therefore needed. Various strategies can be considered to address this problem. Because IPA starts with the inhalation of conidia, one approach is the topical administration of antifungals inside the lungs. We report the results of a study that is the first placebo-controlled trial, to our knowledge, on the use of aerosolized amphotericin B for the prevention of IPA. Inhalation of liposomal amphotericin B reduced
the incidence of IPA from 14% to 4% ($P = .005$). The renal toxicity of intravenous amphotericin B has hindered the prophylactic use of this drug. Inhalation therapy circumvents this problem. Avoiding systemic drug exposure also eliminates the risk of harmful drug-drug interactions. Azoles interfere with CYP P450–mediated metabolism of simultaneously administered drugs. Life-threatening interactions exist, and the observed increase in mortality with the use of itraconazole in patients undergoing allogeneic stem cell transplantation may have been the result of a cyclophosphamide-itraconazole interaction [17]. Also, systemic azole prophylaxis decreases the sensitivity of serum galactomannan monitoring for early diagnosis of IPA [18]. Amphotericin B is not absorbed systemically and therefore is unlikely to have the same effect.

In this study, galactomannan detection in BAL specimens was part of the diagnostic procedure, and galactomannan was found in BAL specimens in 21 of 24 patients with IPA. In many hematology centers, galactomannan detection in BAL specimens is not a standard procedure. The modified EORTC-MSG definition of IPA also includes patients without detectable galactomannan but with a halo or an air-crescent sign on CT. Many investigators consider this radiologic sign sufficient specifici for the diagnosis of IPA [11, 15, 16]. It is reassuring that we observed a significant reduction of IPA when both the original and modified definition of IPA were applied. With the latter, a decrease in the incidence of IPA from 17% to 8% was observed among individuals assigned to receive liposomal amphotericin B treatment. Because inhaled liposomal amphotericin
Amphotericin B may decrease the sensitivity of galactomannan monitoring in BAL, the fact that we observed a significant reduction of IPA with definition not depending on galactomannan detection is important.

In the only published controlled study, to our knowledge, on the use of amphotericin B inhalation for the prevention of IPA, Schwartz et al. [11] found no reduction of IPA incidence with aerosolized amphotericin B treatment. Several reasons may explain the lack of effect in their study. First, pulmonary pharmacokinetics and toxicity of inhaled liposomal amphotericin B used in our study may differ from conventional amphotericin B used by Schwartz and colleagues. In this regard, Griese et al. observed that deoxycholate impairs the surface tension–lowering function of pulmonary surfactant. In contrast, the liposomal carrier of amphotericin B consists of phospholipids and cholesterol and exhibits a surfactant-like function [7]. Also, the inhalation system used in our study was designed to maximize efficacy of inhaled liposomal amphotericin B by targeting peripheral airways through optimal aerosol particle size and optimal lung dose. Finally, the incidence of IPA per neutropenic episode in our population was 9% (13.6% per patient) in the placebo group but only 6% in the study by Schwartz and col-

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**Figure 2.** Kaplan-Meier curves for the occurrence of invasive pulmonary aspergillosis (IPA) according to modified European Organization for Research and the Treatment of Cancer–Mycoses Study Group definitions by randomized group for the intent-to-treat analysis (A) and the on-treatment analysis (B). Numbers along curves denote the number of patients studied at the various episodes. AmB, amphotericin B.
Galactomannan detection in BAL specimens is more sensitive than galactomannan detection in serum specimens and may explain the higher incidence in our study [16, 19, 20].

We observed no systemic toxicity during the study. Creatinine levels in patients after the last inhalation were comparable to baseline levels. Tolerance of inhalation therapy in this ill-patient population was reasonable, but more patients treated with liposomal amphotericin B than with placebo temporarily or completely discontinued therapy. The higher incidence of cough during inhalation therapy was responsible for this difference. A total of 30% of placebo-treated patients discontinued inhalation therapy at some time during the study, which suggests that the inhalation effort per se was an obstacle for some patients. Despite the higher treatment discontinuation rate with liposomal amphotericin B, the ITT analysis, which includes all patients who received at least 1 inhalation, showed a significant reduction in IPA. Additional efforts to increase compliance with the use of inhalation therapy are warranted. Ruigrok et al. [21] previously showed that in a rat model of IPA an improved rate of survival was observed up to 6 weeks after a single amphotericin B inhalation. This finding suggests that less frequent inhalations could still be protective. Recently, the development of an amphotericin B inhalation powder was described. If tolerated well by patients, this may make inhalation therapy easier to administer [22].

Some limitations of the study have to be noted. Allogeneic transplant recipients with graft-versus-host-disease continue to be at a high risk for IPA, but these patients were not included in the study.

This clinical trial was halted after 62 months when 271 of the planned 340 patients had been included. Several reasons led to the decision to prematurely discontinue the study. The recruitment of 69 more patients would have taken a minimum of 16 extra months. Also, in 2005, promising results about the use of posaconazole for the prevention of IPA were presented. At the time the statistical analysis was performed, we discovered a higher incidence of IPA in the placebo group than was projected at the time the study was designed. This higher incidence resulted in >90% power to show the anticipated IPA reduction.

This study was not designed to show a reduction in IPA-related or overall mortality with liposomal amphotericin B inhalation. Therefore, no definite conclusions can be drawn about mortality at this time, although the observed nonsignificant reduction in IPA-related mortality in the OT population from 6% to 1% is encouraging.

In conclusion, the results of this study suggest that inhalation therapy with liposomal amphotericin B may play a role in the prevention of IPA in patients with prolonged neutropenia. Although it was not compared directly to posaconazole and is without documented survival benefit this therapy could present an alternative approach to IPA prevention.

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Potential conflict of interest. All authors: no conflicts.

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