# Liposomal Amphotericin B in Combination With Caspofungin for Invasive Aspergillosis in Patients With Hematologic Malignancies

## *A Randomized Pilot Study (Combistrat Trial)*

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**BACKGROUND.** Invasive aspergillosis (IA) has a poor prognosis in immunocompromised patients. Combinations of drugs that act on different targets are expected to improve the clinical efficacy of separate compounds.

**METHODS.** Patients with proven or probable IA were randomized in a prospective, open pilot study to receive either a combination of liposomal amphotericin B (AmB) at the standard dose (3 mg/kg daily) and caspofungin at the standard dose or monotherapy with a high-dose AmB regimen (10 mg/kg daily).

**RESULTS.** Thirty patients (21 men and 9 women) with hematologic malignancies were analyzed, and there were 15 patients in each arm. The median duration of treatment was 18 days for the combination group and 17 days for the high-dose monotherapy group. At the end of treatment, there were significantly more favorable overall responses (partial or complete responses; $P = .028$) in the combination group (10 of 15 patients; 67%) compared with the high-dose monotherapy group (4 of 15 patients; 27%). Survival rates at 12 weeks after inclusion were 100% and 80%, respectively. Infusion-related reactions occurred in 3 patients in the high-dose monotherapy group. A 2-fold increase in serum creatinine occurred in 4 of 17 patients (23%) who received high-dose monotherapy and 1 of 15


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patient (7%) who received combination therapy; hypokalemia <3 mmol/L occurred in 3 patients and 2 patients, respectively.

**CONCLUSIONS.** The combination of liposomal AmB and caspofungin was promising as therapy for IA compared with monotherapy. A trial that includes more patients will be required next to confirm the results of this pilot study. ***Cancer* 2007;110:2740–6.** © *2007 American Cancer Society.*

**KEYWORDS: invasive aspergillosis, antifungal treatment, drug combination, liposomal amphotericin B, caspofungin.**

Invasive aspergillosis (IA) is a major complication with a high mortality rate in immunocompromised patients.[1] For decades, the recommended treatment for IA has been amphotericin B (AmB) deoxycholate. However, its toxicity led to the development of lipid formulations. In particular, it was demonstrated that the liposomal formulation of AmB (AmBisome) had an improved therapeutic index and a more favorable toxicity profile than AmB deoxycholate or AmB lipid complex forms.[2–4]

Voriconazole recently was approved for first-line therapy in IA. In a large randomized trial, voriconazole showed efficacy and survival benefits over AmB.[5] Caspofungin, which is the first of a new class of antifungal drugs (echinocandins), has been approved as "salvage" therapy for IA.[6] However, despite these therapeutic advances, IA remains associated with high morbidity and an unacceptable rate of mortality. Thus, in patients with acute leukemia and stem cell transplantation, the IA-related mortality rate ranges from 50% to 80%.[7]

The large spectrum of AmB prompted new strategies to improve antifungal treatment. A first approach was to assess higher dosing of liposomal AmB. Indeed, the pharmacodynamic properties, preclinical data from animal models, and response rates of patients who received doses >3 mg/kg per day suggested that liposomal AmB could improve outcomes and survival.[4,8] However the randomized comparative study of Cornely et al.[9] did not demonstrate a greater benefit of the 10 mg/kg daily dose over the standard dose.

The second therapeutic strategy was to combine liposomal AmB with another antifungal drug. The benefits of drug combination have been demonstrated largely in other infectious diseases, such as human immunodeficiency viral infection and tuberculosis. Generally, this strategy needs to associate drugs with different pharmacologic targets.[10] Simultaneous inhibition of fungal cell wall biosynthesis (echinocandins) and disruption of cell membrane integrity (polyenes) may result in synergistic interaction against *Aspergillus spp.* Recent studies in experimental rodent aspergillosis have suggested that the association of AmB and caspofungin decreased tissue infection and increased survival.[11,12]

Despite the frequent clinical use of antifungal combination therapy for primary or salvage therapy of IA in many centers, to date, no randomized study comparing monotherapy with combination therapy has been performed. Case reports and retrospective studies have indicated that the combination of caspofungin with a lipid formulation of AmB or an azole may be beneficial as salvage therapy.[13–18] Marr et al. suggested an improved survival rate with combined voriconazole and caspofungin compared with voriconazole alone.[18] However, their retrospective cohort study compared 2 groups of patients with IA who were treated and assessed during 2 different periods.

The objective of the current study was to compare 2 new strategies of antifungal treatment (high-dose liposomal AmB vs standard-dose liposomal AmB plus caspofungin) based on the assumption that both regimens were more effective than 3 mg/kg per day of liposomal AmB monotherapy. The efficacy results from the study by Cornely et al.,[9] which did not demonstrate a difference between 10 mg/kg per day versus 3 mg/kg per day of liposomal AmB, were not known at the time we initiated the current study.

Herein, we report the results from a prospective, randomized pilot study comparing monotherapy on a high-dose regimen of liposomal AmB (10 mg/kg per day) with a combination of liposomal AmB (3 mg/kg per day) plus caspofungin at the standard dose for initial therapy of IA in immunocompromised patients.

## MATERIALS AND METHODS

### Study Design

This was a national, multicenter, pilot, prospective, randomized open trial (the Combistrat trial) in patients with proven or probable IA according to criteria of the European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG).[19] Patients were randomized (1:1) to receive 3 mg/kg per day liposomal AmB in combination with caspofungin in the "combination group" or 10 mg/kg

per day liposomal AmB in the "high-dose monotherapy group."

The protocol was conducted in accordance with the Declaration of Helsinki and French law for biomedical research and was approved by the Ethics Committee of Dijon (France). Written informed consent was obtained from each patient or from his or her legal guardian before any study procedure. The study has been registered in ClinicalTrials.gov under the identifier NCT00334412.

### Patient Population

Patients were eligible if they were aged ≥10 years, had proven or probable IA according to the EORTC/MSG criteria, and were immunocompromised. Patients were not enrolled if any of the following criteria were met: life expectancy <30 days; allogeneic stem cell transplantation recipient in the 6 previous months; chronic invasive fungal infection (defined as signs or symptoms of invasive fungal infection for >4 weeks before study inclusion); prior antifungal systemic therapy ≥96 hours for the current, documented IA (however, prior systemic antifungal therapy for prophylaxis or as empiric therapy for febrile neutropenia was permissible); use of another investigational, unlicensed drug within 30 days of screening or concurrent participation in another clinical trial; serum creatinine level >2-fold the upper limit of normal; serum alanine or aspartate aminotransferase levels >5-fold the upper limit of normal; pregnant or lactating women; and history of allergy or serious adverse reaction to any polyene antifungal agent or echinochandin derivatives.

A proven IA diagnosis required the identification of typical hyphal elements after histopathologic or cytopathologic examination with evidence of associated tissue damage (either microscopically or unequivocally by imaging) or the growth of *Aspergillus* organisms from a sample obtained by sterile procedure from a normally sterile site and clinically or radiologically abnormal site consistent with infection (excluding urine and mucous membrane). A probable IA diagnosis required at least 1 host factor criterion, and 1 microbiologic criterion, and 1 major (or 2 minor) clinical criteria from abnormal site consistent with infection, and no other pathogen detected to account for the clinical or radiographic signs of infection. In a modification of EORTC/MSG criteria and in accordance with recent studies,[5,9] the diagnosis of probable IA also included patients with recent neutropenia (<500 neutrophils/mm$^3$) within 14 days of study inclusion and a "halo" or "air-crescent" signs on a chest computed tomography (CT) scan.[20] A CT scan diagnosis of IA was verified by a central data review board that included 1 hematologist and 2 radiologists who confirmed IA. Serum galactomannan assay (Platelia; Bio-Rad Laboratories, Hercules, Calif; Bio-Rad, Marnes-la-Coquette, France) with an optical density index of ≥1.0 was considered positive.

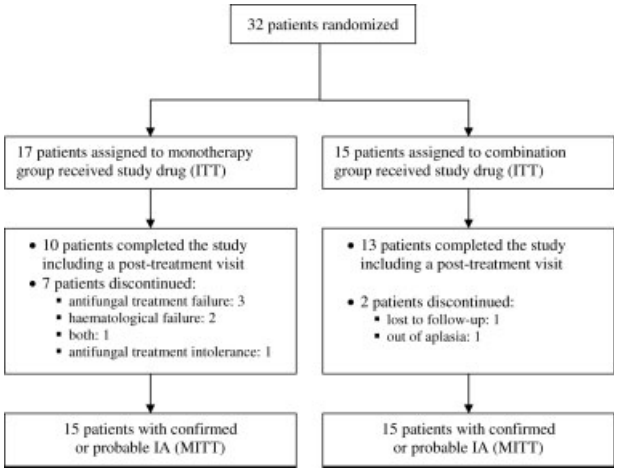


**FIGURE 1.** Disposition of patients. ITT indicates the intent-to-treat population; MITT, the modified intent-to-treat population.

### Administration of Study Drugs

Patients who were randomized to the combination group received a dose of 3 mg/kg per day of intravenous (iv) liposomal AmB (AmBisome) and iv caspofungin (Cancidas) (70 mg on Day 1 followed by 50 mg daily thereafter). Patients in the high-dose monotherapy group received 10 mg/kg per day of liposomal AmB (Fig. 1).

Treatment was administered for at least 14 days; end of treatment (EOT) was defined as the time at which, in the opinion of the investigator, study drug could be discontinued because of at least 1 of the following reasons: resolution of the infection was complete, efficacy could not be improved further, need of drug discontinuation because of toxicity, need of other systemic antifungal agents, or clinical and radiographic evidence of progression of invasive fungal infection. Administration of antipyretic and/or antihistamine medications for prevention of infusion-related reactions was allowed.

### Assessment of Efficacy

A complete response was defined as the complete resolution of all signs and symptoms attributable to the invasive fungal infection compared with baseline and complete or near complete clearing of CT (or magnetic resonance imaging) scan abnormalities

associated with active fungal infection (or persistent residual scarring only) and eradication of the pathogen. A partial response was defined as meaningful improvement of all clinical signs and symptoms attributable to IA and a decrease $\geq$50% in radiographic signs. A stable response was defined as minor or no improvement but no worsening of attributable signs, symptoms, radiographic, and/or bronchoscopic findings. Failure was defined as progression of infection based on an increase in the number and/or severity of clinical signs and symptoms attributable to the IA, worsening of CT scan abnormalities consistent with progressive infection, and persistently positive cultures, histopathologic findings, or *Aspergillus* antigen assays.

Complete (cure) and partial (improvement) overall responses were considered favorable responses. Stable overall response and failures were considered unfavorable responses.

### Statistical Analysis

The primary efficacy population was the modified intent-to-treat (MITT) population, which we defined as patients who received at least 1 dose of randomized treatment and who also had a confirmed diagnosis of proven or probable IA. All patients who received at least 1 dose of randomized treatment were included in the safety population.

The primary efficacy endpoint was the percentage of patients who had favorable overall responses (partial or complete responses) at EOT. Secondary efficacy endpoints included the time to favorable overall response, the time to complete response, survival at EOT, percentage of patients with recurrent infection (defined as failure for overall response), and survival during the 4-week posttreatment follow-up.

The chi-square test was used to compare primary endpoints of the 2 treatment groups. For secondary endpoints, chi-square tests or Cochran-Mantel-Haenszel tests were used for qualitative data, and the Kaplan-Meier method was used to estimate time-to-event endpoints.

## RESULTS

### Patients

In total, 32 patients were enrolled between April 2004 and July 2005 in 9 sites in France. Baseline characteristics of the 30 patients of the MITT population are presented in Table 1. The characteristics of patients at baseline were balanced well between treatment groups; only neutropenia duration before inclusion was significantly longer in the combination group compared with the high-dose monotherapy group ($P = .015$; Wilcoxon's test).

**TABLE 1**
**The Disposition of Patients in the Modified Intend-to-treat Population at Inclusion**

| Characteristic | No. of patients (%) | |
|---|---|---|
| | High-dose monotherapy group (n=15) | Combination group (n=15) |
| Men/women, n | 10/5 | 11/4 |
| Mean age [range], y | 57.3 [25–72] | 49.9 [16–75] |
| Underlying condition | | |
| Acute myeloid leukemia | 10 (67) | 14 (93) |
| Chronic lymphocytic leukemia | 3 (20) | 0 (0) |
| Myeloproliferative disorders | 2 (13) | 0 (0) |
| Acute lymphoid leukemia | 0 (0) | 1 (7) |
| Hematologic status* | | |
| Remission | 4 (27) | 2 (14) |
| Stable disease | 3 (20) | 5 (36) |
| Progressive disease | 8 (53) | 7 (50) |
| Median neutropenia duration before inclusion [range], d | 15 [3–58] | 31.5 [3–115]† |
| Pulmonary invasive aspergillosis | | |
| Proven | 3 (20) | 1 (7) |
| Probable | 12 (80) | 14 (93) |
| Basis of diagnosis‡ | | |
| Halo sign | 10 (67) | 9 (60) |
| Positive galactomannan assay (optical density index $\geq$1)§ | 7 (47) | 5 (33) |
| Bronchoalveolar lavage | | |
| Positive culture | 3 (20) | 1 (7) |
| Positive cytology | 2 (13) | 1 (7) |

* Data were missing for 1 patient in the combination group.

† $P = .015$ (Wilcoxon test): There was no significant difference between treatment groups for the other characteristics.

‡ In addition to neutropenia, computed tomography scan, and clinical signs.

§ Two successive positive samples.

Two patients did not meet the criteria for a diagnosis of proven or probable IA according to the central data review board (nonspecific signs of IA on CT scan) and were disqualified for efficacy analysis (MITT). One patient was a protocol exception who was validated by the central data review board: His diagnosis of IA was established by the investigator but was not in accordance with the EORTC/MSG criteria. Indeed, this patient had no fever, neutropenia duration was <10 days, and he did not receive corticoids during the last month; however, he received another immunosuppressant treatment, was positive for Aspergillus antigenemia, and had a chest CT scan with signs that were suggestive of pulmonary IA.

Two patients in the combination group had an allograft >6 months before their inclusion (1 patient had cutaneous and mucous graft-versus-host disease at inclusion). All patients presented with pulmonary IA.

**TABLE 2**
**Study Drug Administration and Antifungal Drugs Received After End of Treatment (Modified Intend-to-treat Population)**

| Parameter | No. of patients (%) | |
|---|---|---|
| | High-dose monotherapy group (n=15) | Combination group (n=15) |
| Median time on study drug [range], d | 17 [4–24] | 18 [10–35] |
| Median adherence [range], %* | 100 [52–101] | 100 [97–100] for AmB; 100 [94–136] for Caspo |
| Antifungal drugs received after EOT | | |
| Any systemic antifungal treatment | 13/15 (87) | 14/15 (93) |
| Intravenous or oral voriconazole monotherapy | 10 (78) | 13 (93) |
| Combinations | 3 (22) | 1 (7) |
| Voriconazole plus caspofungin | 2 | 0 |
| Voriconazole plus caspofungin plus liposomal AmB | 1 | 1 |

AmB indicates amphotericin B; Caspo, caspofungin; EOT, end of treatment.
* Total drug received/total drug expected)×100.

**TABLE 3**
**Efficacy of Antifungal Treatment (Modified Intend-to-treat Population)**

| Parameter | No. of patients (%) | |
|---|---|---|
| | High-dose monotherapy group (n=15) | Combination group (n=15) |
| Overall treatment response at EOT | | |
| Favorable | 4 (27) | 10 (67)* |
| Complete response | 0 | 0 |
| Partial response | 4 | 10 |
| Unfavorable | 11 (73) | 5 (33) |
| Stable response | 6 | 4 |
| Failure | 4 | 1 |
| Missing value | 1 | 0 |
| Time to favorable treatment response, d | 4 | 10 |
| Mean ± SD | 14 ± 0.8 | 19.3 ± 9.3 |
| Median [range] | 14 [13–15] | 14 [10–38] |
| Survival at EOT | 14 (93) | 15 (100) |
| Survival at Wk 12 | 12 (80)† | 15 (100) |

EOT indicates end of treatment; SD, standard deviation.
* $P = .028$ (favorable response high-dose monotherapy group vs combination group; chi-square test).
† Three deaths were caused by progression of the underlying hematologic condition (for 1 of those patients, fungal infection contributed to the death according to the investigator).

## Duration of Therapy

The median duration of study drug administration was 17 days and 18 days in the high-dose monotherapy and combination groups, respectively (Table 2). Treatment adherence (total drug received/total drug expected × 100) was near 100%. The majority of patients received antifungal therapy after EOT (90%), mostly voriconazole monotherapy (iv or oral), without differences between treatment groups.

## Outcomes

The MITT analysis indicated that the overall response at EOT was significantly ($P = .028$) more favorable for patients in the combination group (10 of 15 patients; 67%) compared with patients in the high-dose monotherapy group (4 of 15 patients; 27%) (Table 3). At Week 12, a favorable response was obtained by 10 of 15 patients in the high-dose monotherapy group (67%; 8 patients had a partial response and 2 patients had a complete response) and by 12 of 15 patients in the combination group (80%; 9 patients had a partial response and 3 patients had a complete response).

A favorable or unfavorable response at EOT was independent of hematologic status at EOT (recurrence, remission, or stable; $P = .442$; Fisher exact test). The survival rate at EOT was 97% (1 death had occurred in the high-dose monotherapy group). At Week 12, all 15 patients in the combination group were alive, whereas 3 of 15 patients had died in the high-dose monotherapy group. Those 3 patients died because of progression of the underlying hematologic condition; and, in 1 patient, fungal infection contributed to the death according to the investigator.

## Safety

All randomized patients received at least 1 dose of study treatment and were assessable for safety analysis. Study drug-related adverse events (n = 28 events) were less frequent in the combination group (n = 4 events) than in the high-dose monotherapy group (n = 24 events). Infusion-related reactions occurred in 3 patients in the high-dose monotherapy group (flush, cervical or thoracic pain, chills, and nausea).

Twelve serious adverse events were reported for 11 patients; 3 patients died because of aggravation of underlying conditions with no relation to study drug toxicity. Only 1 serious adverse event (renal disorder) was related to study drug (high-dose monotherapy group).

An analysis of biologic parameters indicated that a 2-fold increase in serum creatinine levels occurred in 4 of 17 patients (23%) in the high-dose monotherapy group and 1 of 15 patients (7%) in the combination group. Three patients in the monotherapy group and 2 patients in the combination therapy group had hypokalemia <3 mmol/L at least once during the study.

## DISCUSSION

IA remains a serious condition, and the treatments that have been evaluated to date have been based on conventional strategies. Recently marketed antifungal agents have made the concept of drug combination possible. Until now, antifungal drug combinations have been assessed only as add-on treatment in salvage therapy. To our knowledge, the current study represents the first prospective randomized study that was designed to compare monotherapy versus combination therapy as first-line antifungal therapy in patients with IA.

With liposomal AmB, which remains the antifungal drug with the largest spectrum, 2 therapeutic strategies have been explored: dosage increase to 10 mg/kg per day and combination with another antifungal drug. It was reported recently by Cornely et al. that no additional efficacy benefit was obtained with AmB 10 mg/kg per day in patients with IA.[9] It has been suggested in case reports and retrospective studies[13–18] that the combination of caspofungin and a lipid formulation of AmB or an azole may be beneficial for patients with IA who fail on conventional antifungal first-line monotherapy.

The current randomized study in hematologic immunocompromised patients with IA demonstrated that the combination of liposomal AmB (3 mg/kg per day) and caspofungin led to a better favorable overall response rate (67% vs 27%; $P = .028$) than monotherapy with liposomal AmB alone (high-dose regimen; 10 mg/kg per day) at EOT (primary criterion). This favorable outcome was accompanied by high survival rates (100% vs 93% at EOT, respectively). Although the difference was not statistically significant, this difference favoring combination therapy also was observed for patient survival at 12 weeks (100% survival vs 80% in the high-dose monotherapy group).

It could be argued that the duration of neutropenia differed significantly between the treatment groups. Indeed, patients with longer neutropenia duration have generally less favorable outcomes, as evidenced, for example, in the AmBiLoad study, which included both patients with and without neutropenia.[9] The baseline difference for neutropenia duration in our study did not change the conclusion. On the contrary, this difference confirmed the interest of the antifungal drug combination, because patients who were supposed to have a poor outcome were in the group with a better outcome.

A major concern with combination antifungal drug therapy is the risk of increased toxicity, particularly renal toxicity. Retrospective studies or case reports have suggested a good safety profile for combination antifungal drugs. This also was true in our prospective trial, in which only a single serious adverse event was related to the study regimen.

This study presents some limitations. First, it was a pilot study with a small number of patients. Large, prospective, randomized studies certainly are required but are difficult to perform, because IA is relatively infrequent; and, until recently, only low gains in outcomes were expected. Thus, a recent editorial estimated that, to show a 10% improvement in survival over what is achieved with voriconazole alone most likely will require 570 evaluable patients.[21] Thus, pilot studies are necessary to carefully design larger prospective studies. Nevertheless, the sample size argument could be reversed, and it could be argued that, despite the low statistical power of the current study design, the statistically significant difference between treatment groups emphasizes the efficacy of the drug combination.

Another limitation was the open-label nature of the trial, which may have affected the results. However, the study was based in centers that had extensive experience with IA management. Inappropriate outcome evaluations are unlikely to have occurred on a scale large enough to change the conclusions of the study. Furthermore an expected superiority of 1 regimen over the other was not obvious for the investigators (the results from the Ambiload study[9] were not known when this study was performed).

Single-drug therapy, as either first-line or salvage treatment, rarely obtains response rates greater than 50% to 60%. Combination therapy is an attractive concept for treating invasive mycoses. Optimal combination regimens remain unclear. However, given its broad spectrum encompassing difficult to treat patients, we believe that liposomal AmB is the drug of choice for invasive mycosis combination therapy.

In conclusion, the results of the current study suggest that the combination of liposomal AmB and caspofungin therapy is promising for improved efficacy in patients with IA. Next, a trial that includes more patients will be required to confirm our results.

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