MAJOR ARTICLE

A Randomized, Blinded, Multicenter Trial of Lipid-Associated Amphotericin B Alone versus in Combination with an Antibody-Based Inhibitor of Heat Shock Protein 90 in Patients with Invasive Candidiasis

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(See the editorial commentary by Casadevall on pages 1414–6)

Background. Mycograb (*Neu*Tec Pharma) is a human recombinant monoclonal antibody against heat shock protein 90 that, in laboratory studies, was revealed to have synergy with amphotericin B against a broad spectrum of *Candida* species.

Methods. A double-blind, randomized study was conducted to determine whether lipid-associated amphotericin B plus Mycograb was superior to amphotericin B plus placebo in patients with culture-confirmed invasive candidiasis. Patients received a lipid-associated formulation of amphotericin B plus a 5-day course of Mycograb or placebo, having been stratified on the basis of *Candida* species *(Candida albicans* vs. non-*albicans* species of *Candida)*. Inclusion criteria included clinical evidence of active infection at trial entry plus growth of *Candida* species on culture of a specimen from a clinically significant site within 3 days after initiation of study treatment. The primary efficacy variable was overall response to treatment (clinical and mycological resolution) by day 10.

Results. Of the 139 patients enrolled from Europe and the United States, 117 were included in the modified intention-to-treat population. A complete overall response by day 10 was obtained for 29 (48%) of 61 patients in the amphotericin B group, compared with 47 (84%) of 56 patients in the Mycograb combination therapy group (odds ratio [OR], 5.8; 95% confidence interval [CI], 2.41–13.79; $P < .001$). The following efficacy criteria were also met: clinical response (52% vs. 86%; OR, 5.4; 95% CI, 2.21–13.39; $P < .001$), mycological response (54% vs. 89%; OR, 7.1; 95% CI, 2.64–18.94; $P < .001$), *Candida*-attributable mortality (18% vs. 4%; OR, 0.2; 95% CI, 0.04–0.80; $P = .025$), and rate of culture-confirmed clearance of the infection (hazard ratio, 2.3; 95% CI, 1.4–3.8; $P = .001$). Mycograb was well tolerated.

Conclusions. Mycograb plus lipid-associated amphotericin B produced significant clinical and culture-confirmed improvement in outcome for patients with invasive candidiasis.

Candidemia remains a costly burden to health care [1]. *Candida*-attributable mortality rates range from 4.4%–8.7% in noninferiority trials of conventional amphotericin B [2, 3] to 10% in observational studies [4] and 15%–49% in case-matched studies [5–7]. Lipid-associated formulations of amphotericin B (L-ampho-

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tericin), such as Abelcet (Zeneus) or Ambisome (Gilead), are preferable to conventional amphotericin B, because the lipid-associated formulations have a superior safety profile, such that the efficacy of amphotericin B is not distorted by treatment withdrawals resulting from its toxicity [8].

The poor outcome for monotherapy and the availability of new antifungals [8, 9] have kindled interest in combination therapy [10, 11]. Fluconazole plus 5–6 days of conventional amphotericin B treatment, compared with fluconazole alone, has been associated with a trend toward greater success [12].

In the present study of adults with culture-confirmed invasive candidiasis, the objective was to compare L-amphotericin alone and in combination with Mycograb (*Neu*Tec Pharma), a human recombinant monoclonal antibody targeting heat shock protein 90 (hsp90). Hsp90 is a molecular chaperone present in the fungal cell wall and extracellular material [13] and has been described as the "Achilles' heel" of fungi [14]. An hsp90 inhibitor (geldanamycin) was found to increase the susceptibility of *Candida* and *Aspergillus* species to fluconazole and caspofungin, respectively, in vitro [15]. The presence of endogenous antibody to hsp90 closely correlated with recovery in patients with invasive candidiasis treated with amphotericin B [16]. Mycograb mimics this naturally occurring inhibitor of hsp90 and is thus a logical partner in combination therapy. Mycograb has demonstrated synergy against a broad range of *Candida* species in combination with amphotericin B in vitro and in animal models of invasive candidiasis [13], and different antibodies against the same hsp90 epitope were protective in murine candidiasis [17, 18].

Researchers in studies with single antifungal products have not reached a consensus on treatment duration and study end points [19]. If a combination of drugs is more effective than the single agent, then there should be an improvement in the clinical and mycological responses in recipients of combination therapy at a fixed time representing the minimum duration of monotherapy. The median duration of therapy for Abelcet and Ambisome is 10–13 days [20–22]. Therefore, the primary time selected for this study was day 10. The primary variable was composite, requiring clinical and culture-confirmed resolution of candidiasis. The interpretation of clinical response is more subjective if it includes patients who show improvement, as well as patients who experience cure; thus, patients who had a partial response were classified among the nonresponders. Secondary end points included the rate of mycological clearance over time (removing the limitations of a fixed time point) and *Candida*-attributable mortality 4 weeks after receipt of the last dose of study drug.

Restriction to patients with candidemia was considered to be inappropriate, because this restriction misses 50% of necropsy-proven cases (probably more if receiving fluconazole prophylaxis) and potentially leads to an overrepresentation of less-virulent yeasts, such as *Candida parapsilosis* [2, 23]. Severely ill patients (who are often excluded from investigational studies because of renal or hepatic dysfunction, an estimated duration of survival of <5 days, or failure of prior antifungal therapy) were included in this trial [2, 3, 24].

PATIENTS AND METHODS

Study design. The protocol was reviewed by the relevant ethics committees or institutional review boards; written informed consent was obtained from each patient or legal representative before enrollment, and applicable guidelines for human studies were followed. The trial was conducted in 10 European countries and in the United States during the period of December 2002 through April 2004, and patients from 26 institutions were recruited.

Enrollment. To be enrolled, patients had to be ⩾18 years of age and to have had ⩾1 positive *Candida* culture of a specimen from a clinically significant site within the previous 3 days, as well as at least 1 of the following signs at study entry: hyperthermia (temperature, >38°C), hypothermia (temperature, <36°C), tachycardia (heart rate, >110 beats/min), hypotension (mean blood pressure, <70 mm Hg), high WBC count (i.e., >11,000 cells/mm^3), left shift, need for vasopressor support, or other abnormalities consistent with an ongoing infectious disease process. Cultures of samples from significant sites or specimens included blood cultures and/or cultures of samples from a deep, normally sterile site. Respiratory secretions, oropharyngeal specimens, and esophageal specimens were not considered to be significant, and positive culture results from these sites were not considered to be indicative of invasive candidiasis. Patients with endocarditis were excluded.

Study procedures. Patients were stratified into groups on the basis of *Candida* species *(Candida albicans* vs. non-*albicans* species of *Candida)* and were randomly assigned to receive either intravenous Mycograb (1 mg/kg) or placebo (saline) every 12 h for 5 days. In addition, each patient was treated with the manufacturer's recommended dose of either Abelcet (5 mg/kg per day) or Ambisome (3 mg/kg per day) for a minimum of 10 days. Patients and investigators remained blinded throughout the study. Apart from systemic antifungal therapy, no other concomitant medications were censored.

Both mycological and clinical responses were used in the assessment of efficacy. The study drug was given on days 1–5, and samples were obtained for culture on days 2, 3, 4, 5, 6, 8, and 10 or until the signs and symptoms of infection had resolved and culture results were repeatedly negative. Clinical response to treatment was assessed on days 4, 5, 6, 8, 10, and 33, and the course of the disease over the previous 24 h was assessed on a daily basis up until day 10. The assessment of clinical response was made by the local investigator, and the response was considered to be complete if all signs and symptoms thought to be

Table 1. Characteristics of patients enrolled and included in the analysis of efficacy (i.e., the modified intention-to-treat [ITT] population) and analysis of safety (i.e., the safety population).

Group or finding	No. of patients who received lipid-associated amphotericin B	
	Plus placebo (n = 71)	Plus Mycograb (n = 68)
No study drug given	2	0
Safety population		
No. of patients	69	68
No positive *Candida* culture result[a]	6	8
No evidence of sepsis[b]	2	0
Indeterminate evaluation findings[c]	0	3
Candida endocarditis	0	1
Modified ITT population		
No. of patients	61	56
Symptomatic candiduria	6	7
Deep abdominal drain	1	2
Infected necrotomy site	1	3
Subgroup analyses		
Candidemia and/or positive sterile site culture results[d]	53	44
Candidemia	35	33
Positive sterile site culture results[e]	32	23

NOTE. Mycograb (*Neu*Tec Pharma) is an antibody-based inhibitor of heat shock protein 90.

[a] No positive result of *Candida* culture of a sample from any site within 14 days of study entry (3 patients), or the only positive culture result was from a respiratory secretion (10 patients) or sample from an insignificant site (1 patient).

[b] No clinical or laboratory evidence of sepsis at the time of study entry for patients who were not immunosuppressed.

[c] Patients died (2 patients) or were withdrawn from the study (1 patient) on the first day of study treatment, before posttreatment assessments were available.

[d] Positive results of cultures of samples from a deep, normally sterile site and/or positive blood culture results.

[e] Positive results of cultures of samples from a deep, normally sterile site.

due to *Candida* infection had resolved. An independent expert and a safety monitoring committee monitored the trial's safety during the study. Investigators assessed the relationship of adverse events to Mycograb and L-amphotericin.

Evaluation of efficacy. The primary efficacy end point was overall response to treatment on day 10, which was 5 days after receipt of the last dose of study drug and was the minimum duration of therapy with L-amphotericin. A favorable overall response was defined as a complete clinical and mycological response, with resolution of all signs and symptoms of candidiasis and culture-confirmed eradication of the pathogen. Partial improvement, lack of progress, or worsening of the candidiasis were classified as unfavorable.

Secondary efficacy end points included clinical response at day 10, mycological response at day 10, rate of mycological clearance of the infection, and *Candida*-attributable mortality 4 weeks after receipt of the last dose of study drug (i.e., day 33). *Candida*-attributable mortality was defined as a fatality in which the investigator stated that candidiasis significantly contributed to death and for which there was clinical evidence of persistent candidiasis, autopsy evidence, and/or death within 48 h after a positive blood culture result [4].

Statistical analysis. Sample size was determined after a pilot study using nQuery 6 (Statistical Solutions). There was one comparison of interest; therefore, the 5% significance level was not adjusted. A hierarchical test procedure was applied to secondary efficacy variables. The rate of mycological clearance used the time from the baseline positive culture result to the last positive culture result, and the analysis was censored if the last positive culture was not observed because the patient was withdrawn from the study or died.

The prespecified study populations for analysis were the safety population (i.e., all patients who received $\geq$1 dose of study drug) and the modified intention-to-treat (ITT) population (for analysis of efficacy). The modified ITT population included all patients who had culture-confirmed invasive candidiasis and who received at least 1 dose of the study drug and had at least 1 poststudy treatment assessment.

Table 2. Baseline characteristics of patients in the modified intention-to-treat population.

Characteristic	Lipid-associated amphotericin B	
	Plus placebo (n = 61)	Plus Mycograb (n = 56)
Sex		
Male	35 (57)	42 (75)
Female	26 (43)	14 (25)
Age, median years (range)	64 (19–88)	58 (21–76)
Underlying condition or risk factor		
APACHE II score >18	26 (43)	27 (48)
Stay in an intensive care unit	51 (84)	51 (91)
Charlson weighted index of comorbidity	2.92 ± 1.96	3.42 ± 1.88
Smoking[a]	10 (16)	23 (41)
Alcoholism	1 (2)	4 (7)
Chronic obstructive pulmonary disease	7 (11)	10 (18)
Intubation	31 (51)	34 (61)
Liver failure	15 (25)	14 (25)
Renal failure	24 (39)	23 (41)
Diabetes mellitus	8 (13)	10 (18)
Vasopressor support requirement	30 (49)	33 (59)
Neutropenia or AIDS[b]	6 (10)	1 (2)
Corticosteroid use	8 (13)	9 (16)
Other immunosuppression or receipt of chemotherapy	3 (5)	1 (2)
Prior antimicrobial use	53 (87)	48 (86)
Cancer	10 (16)	11 (20)
Receipt of total parental nutrition	10 (16)	12 (21)
Urinary catheter in place	51 (83)	50 (89)
Hemodialysis/peritoneal dialysis	9 (15)	9 (16)
Infection with species other than *Candida parapsilosis*	58 (95)	55 (98)
APACHE II score		
Mean ± SD	17.5 ± 6.7	18 ± 7.9
Range	4–32	3–39
Prior receipt of antifungal therapy for ⩾4 days		
No. (%) of patients	5 (8)	7 (13)
Duration, mean days (range)	9 (4–21)	8 (4–17)
Candida species		
Candida albicans	39 (65)	35 (63)
Candida glabrata	4 (7)	6 (11)
Candida tropicalis	3 (5)	2 (4)
Candida parapsilosis	3 (5)	1 (2)
Candida krusei	2 (3)	0
Unidentified non-*albicans* species	0	2 (4)
Multiple species[c]	9 (15)	10 (18)

NOTE. Data are no. (%) of patients, unless otherwise indicated. Mycograb (*Neu*Tec Pharma) is an antibody-based inhibitor of heat shock protein 90.

[a] Statistically significant ($P < .005$).

[b] Neutropenia was defined as a neutrophil count $<0.5 \times 10^9$ cells/L. The patient with AIDS had a CD4 cell count $<0.2 \times 10^9$ cells/L.

[c] In each case, this included *C. albicans,* with the most common combination being *C. albicans* and *C. glabrata* (6 patients in each group).

Table 3. Assessment of efficacy in the modified intention-to-treat population.

Variable	No. (%) of patients who received lipid-associated amphotericin B		OR ± SE	95% CI	*P*
	Plus placebo (*n* = 61)	Plus Mycograb (*n* = 56)			
Primary efficacy variable					
Complete overall response by day 10	29 (48)	47 (84)	5.762 ± 1.561	2.408–13.787	<.001
Supportive analyses[a]	29 (48)	47 (84)	6.960 ± 1.273	2.698–17.954	<.001
Receipt of Abelcet or Ambisome	29 (48)	47 (84)	5.846 ± 1.251	2.428–14.077	<.001
Secondary efficacy variable					
Complete clinical response by day 10	32 (52)	48 (86)	5.437 ± 1.584	2.207–13.393	<.001
Mycological response by day 10	33 (54)	50 (89)	7.071 ± 1.653	2.640–18.938	<.001
Candida-attributable mortality by day 33	11 (18)	2 (4)	0.168 ± 2.211	0.036–0.797	.025

NOTE. Mycograb (*Neu*Tec Pharma) is an antibody-based inhibitor of heat shock protein 90. Abelcet (Zeneus) and Ambisome (Gilead) are both lipid-associated formulations of amphotericin B.

[a] Supportive analyses used the following covariates as factors in the analysis: *Candida albicans* infection, polyfungal infection, and infection with non-*albicans* species of *Candida*.

A statistical analysis plan was created prior to unblinding by Hartington Statistics and Data Management (London, UK), who conducted all prespecified statistical analyses. All binary outcomes are expressed as ORs with 95% CIs and were analyzed using logistic regression. The speeds of mycological response were determined by Cox proportional hazards regression and Kaplan-Meier plot and are expressed as hazard ratios (HRs) with 95% CIs.

RESULTS

Baseline characteristics. Of 139 patients enrolled, 117 were included in the modified ITT population (table 1). Comparison of baseline characteristics (table 2) revealed that there were no significant differences in APACHE II scores or in the use of Abelcet and Ambisome (87% of patients in each treatment group received Abelcet). The management of central venous catheters did not differ between the 2 groups, and the catheter was removed if it was the source of infection [25]. Infected catheter tips were reported in 12 patients in each group (in the Mycograb group, catheters were infected with *C. albicans* for 9 patients, *Candida glabrata* for 2, and *Candida tropicalis* for 1; in the placebo group, catheters were infected with *C. albicans* for 6 patients, *C. glabrata* for 1, *C. tropicalis* for 2, *Candida parapsilosis* for 2, and *Candida krusei* for 1).

Prespecified efficacy analyses. The primary efficacy variable showed a highly statistically significant difference in favor of combination therapy with Mycograb: only 48% of patients in the placebo group had a complete clinical and mycological response by day 10, compared with 84% of patients who received combination therapy ($P < .001$). Two supportive analyses were performed with respect to species and L-amphotericin (table 3). Efficacy was consistent between sites.

Clinical response was determined to be complete in 86% of Mycograb recipients by day 10, compared with 52% of patients who received placebo ($P < .001$). Mycological resolution was achieved in 89% of Mycograb recipients, compared with 54% of those in the placebo arm ($P < .001$). The rate of mycological clearance (by Kaplan-Meier plot) (figure 1) was more than twice as fast for combination therapy than for amphotericin B alone (HR, 2.3; 95% CI, 1.4–3.8; $P = .001$). The *Candida*-attributable mortality rate decreased from 18% to 4% among patients receiving Mycograb ($P = .025$). Most (12 of 13) *Candida*-attributable deaths occurred by day 12. Overall mortality in the modified ITT population showed a trend in favor of combination therapy at day 12 (16% vs. 21%). Most of the deaths in the placebo group involved patients with APACHE II scores ≤ 25 and were due to *Candida* infection (figure 2). In contrast, most frequently, deaths in the Mycograb group involved patients with APACHE II scores ≥ 25, and the most common cause of death was bacterial sepsis, followed by cancer.

Patient subgroup analyses. Subgroup analyses of responses at day 10 for patients with candidemia, a positive culture result for a normally sterile site, or both revealed statistically significant differences in primary and secondary efficacy variables (table 4). The positive culture results for normally sterile sites included results for ≥ 1 of the following cultures: cultures of specimens of intravenous line tips ($n = 24$), ascite specimens ($n = 9$), laparotomy biopsy specimens ($n = 17$), abdominal wound specimens (with positive blood or urine culture results; $n = 3$), and dialysis catheters ($n = 1$) and deep cultures of specimens from a thoracic wound ($n = 1$), pleural space ($n = 1$), and aortic root prosthesis ($n = 1$) or from an autopsy ($n = 1$). Many (27.4%) of these patients also had ≥ 1 positive urine culture result. The rate of mycological cure was again more than twice as fast for combination therapy for each subgroup (for patients with candidemia, the HR was 2.4 [95% CI,

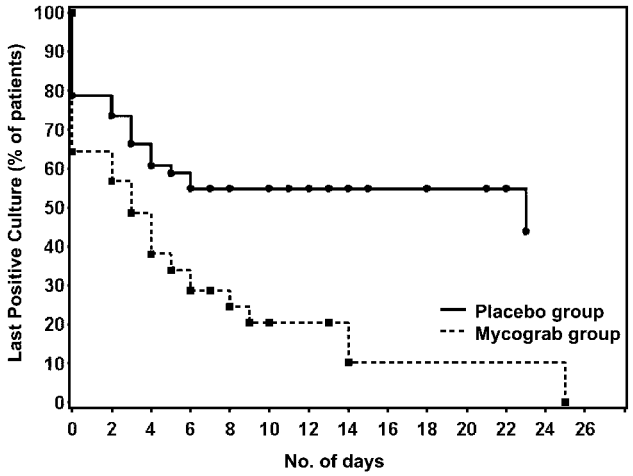

Figure 1. Kaplan-Meier plot of time to last positive culture result for patients receiving lipid-associated amphotericin B plus placebo (placebo group) or plus Mycograb (Mycograb group). Mycograb (*Neu*Tec Pharma) is an antibody-based inhibitor of heat shock protein 90.

1.2–4.7; $P = .011$]; for patients with positive sterile site culture results, the HR was 2.6 [95% CI, 1.2–5.8; $P = .020$]; for patients with candidemia and/or positive sterile site culture results, the HR was 2.6 [95% CI, 1.4–4.6; $P = .002$]). The overall mortality rate on day 12 for patients with candidemia in the Mycograb arm (5 [15%] of 33 patients) was approximately one-half that of the placebo arm (10 [29%] of 35 patients), but this difference decreased over time (on day 33, it was 13 [39%] of 33 Mycograb recipients and 15 [43%] of 35 placebo recipients; by 3 months, it was 48% for both groups [16 of 33 and 17 of 35 patients, respectively]).

A subanalysis of nonneutropenic, HIV-negative patients who were receiving high-dose steroid therapy for preexisting autoimmune disease, colitis, adrenal insufficiency, respiratory insufficiency complicating chronic obstructive pulmonary disease, or acute respiratory distress syndrome or as part of immunosuppression after transplantation revealed that 9 of 9 patients in the Mycograb arm and 4 of 8 in the placebo arm had a complete response on day 10 ($P = .0294$), and 2 cases of *Candida*-attributable mortality occurred in the placebo group. There were 7 patients with baseline neutropenia or AIDS, and the low number and unequal distribution of these patients and the improvement in neutropenia that occurred in 3 of them during the initial 5 days of therapy prevented a meaningful statistical analysis.

For patients who entered the study for whom ⩾4 days of prior treatment with systemic antifungals had failed (table 2), those in the placebo group were less likely to recover (1 [20%] of 5 patients) than were those in the Mycograb group (4 [57%] of 7 patients). There were 2 *Candida*-attributable deaths in the placebo group.

To identify differences in both the number and the severity of underlying pathologies (table 2), the Charlson weighted index of comorbidity was calculated for each patient [26]. The higher the assigned weight for the disorder, the greater the risk of death. In both groups, patients who died had a higher Charlson weighted index than did survivors (for the placebo group, 3.66 ± 1.88 vs. 2.43 ± 1.89 [$P = .0166$]; for the Mycograb group, 4.92 ± 3.46 vs. 2.41 ± 1.84 [$P = .0023$]). In contrast, in the placebo group, the mean Charlson weighted index for patients who had *Candida*-attributable mortalities (3.09 ± 2.2) was similar to that for survivors, which is consistent with candidiasis—rather than underlying pathologies—being responsible for death ($P = .38$).

Recurrences, relapses, and additional antifungal therapy. Persistent isolations (i.e., isolations of the same species in association with the same infection) after day 10 were more common in the placebo group (13 [21%] of 61 patients) than in the combination therapy group (4 [7%] of 56 patients). The incidence of new infection (i.e., infection with a different species or, if the same species, after ⩾2 negative culture results and a re-

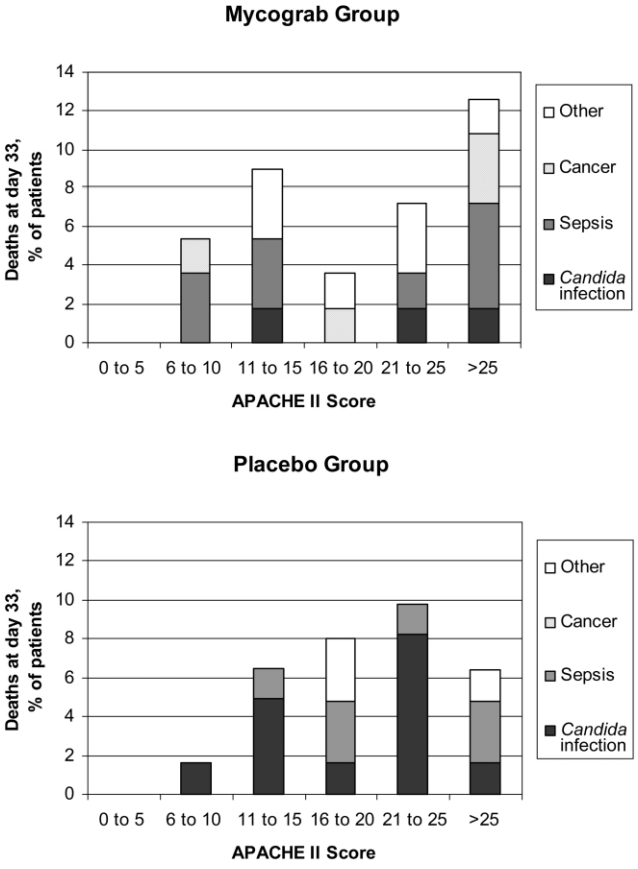

Figure 2. Overall distribution of mortality rates (4 weeks after end of the study drug treatment regimen) and APACHE II score distribution for patients receiving lipid-associated amphotericin B plus placebo (placebo group) or plus Mycograb (Mycograb group). Mycograb (*Neu*Tec Pharma) is an antibody-based inhibitor of heat shock protein 90.

Table 4. Subgroup analyses of candidemia and other forms of invasive candidiasis.

End point, group[a]	Proportion of patients (%) who received lipid-associated amphotericin B: Plus placebo (n = 61)	Plus Mycograb (n = 56)	OR ± SE	95% CI	P
Overall response by day 10					
Candidemia	15/35 (43)	27/33 (82)	6.000 ± 1.761	1.979–18.194	.002
Positive sterile site culture	12/32 (38)	18/23 (78)	6.000 ± 1.866	1.767–20.369	.004
Candidemia and/or positive sterile site culture	21/53 (40)	35/44 (80)	5.926 ± 1.596	2.370–14.814	<.001
Clinical response by day 10					
Candidemia	18/35 (51)	27/33 (82)	4.250 ± 1.758	1.407–12.837	.010
Positive sterile site culture	13/32 (41)	19/23 (83)	6.942 ± 1.930	1.914–25.180	.003
Candidemia and/or positive sterile site culture	24/53 (45)	36/44 (82)	5.437 ± 1.614	2.129–13.888	<.001
Mycological response by day 10					
Candidemia	18/35 (51)	29/33 (88)	6.845 ± 1.880	1.985–23.600	.002
Positive sterile site culture	13/32 (41)	20/23 (87)	9.744 ± 2.047	2.394–39.657	<.001
Candidemia and/or positive sterile site culture	25/53 (47)	38/44 (86)	7.092 ± 1.679	2.568–19.586	<.001
Candida-attributable mortality					
Candidemia	9/35 (26)	2/33 (6)	0.186 ± 2.284	0.037–0.940	.042
Positive sterile site culture	6/32 (19)	0/23 (0)		−32 to −5	.035[b]
Candidemia and/or positive sterile site culture	11/53 (21)	2/44 (5)	0.182 ± 2.223	0.038–0.871	.033
Overall mortality by day 12					
Candidemia	10/35 (29)	5/33 (15)	0.446 ± 1.846	0.134–1.484	.188
Positive sterile site culture	6/32 (19)	4/23 (17)	0.912 ± 2.039	0.226–3.687	.897
Candidemia and/or positive sterile site culture	12/53 (23)	8/44 (18)	0.759 ± 1.666	0.279–2.065	.589

NOTE. Mycograb (*Neu*Tec Pharma) is an antibody-based inhibitor of heat shock protein 90.

[a] These 2 groups overlapped; patients with candidemia may also have had a positive culture result for a sample from a normally sterile site, and vice versa.

[b] Determined by Fisher's exact test for Mycograb versus placebo.

corded complete clinical response for the original, treated infection) was comparable in both groups (7 patients each).

The median (10 days) and mean (10.5 ± 5.6 and 10.0 ± 4.4 days for placebo and Mycograb groups, respectively) duration of L-amphotericin treatment were similar in both groups; however, when premature deaths attributable to *Candida* infection were excluded, the mean values were 11.48 ± 5.6 and 10.05 ± 4.4 days, respectively. Continuation of systemic therapy with another antifungal (usually fluconazole) was more common in the placebo group, increasing the median duration of treatment to 15.0 days, whereas the duration remained 10 days in the Mycograb group. The mean duration of treatment for the placebo group was 17.8 ± 11.3 days, compared with 12.8 ± 7.6 days for the Mycograb group (P = .0061).

This is consistent with a sensitivity analysis performed on the primary efficacy variable, in which, to be classed as having experienced a favorable response, patients also had to discontinue all use of systemic antifungals on day 10. This analysis revealed a highly statistically significant difference between the 2 groups: 52% of patients in the Mycograb group had a complete clinical and mycological response and discontinued systemic antifungal treatment at day 10, compared with 26% of patients in the placebo group (P = .005).

Safety profile. Adverse events that investigators thought may have been causally related to receipt of the study drug occurred in 7 (10%) of Mycograb-treated patients and 5 (7%) of the placebo-treated patients. In the Mycograb-treated group, these events included back pain and vomiting (n = 1); generalized rash (n = 1); hypotension and skin blister (n = 1); hypertension (n = 1); septic shock (n = 1); chills, tachycardia, and worsening pyrexia (n = 1); and low monocyte count (n = 1). Temporary, infusion-related back pain also occurred in 1 patient in the pilot study (data not shown). Adverse events thought by the investigator to be causally related to L-amphotericin treatment were less common in the Mycograb-treated group (table 5).

Comparison of the frequency of all adverse events revealed that episodes of hypertension occurred more frequently in the Mycograb group than in the placebo group (5 [7.4%] of 68 patients vs. 2 [2.9%] of 69 patients; P = .27) and usually occurred within 2 h after receipt of the first dose of Mycograb (4 [6%] of 68 patients).

Table 5. Adverse events believed by the investigator to be causally related to use of Mycograb or lipid-associated amphotericin B.

Adverse event	Related to Mycograb use (n = 68)	Related to lipid-associated amphotericin B use	
		Mycograb group (n = 68)	Placebo group (n = 69)
Clinical event			
Back pain	1 (1.5)	…	…
Vomiting	1 (1.5)	2 (2.9)	1 (1.4)
Rash	1 (1.5)	1 (1.5)	1 (1.4)
Skin blister	1 (1.5)	…	…
Hypotension	1 (1.5)	1 (1.5)	2 (2.9)
Hypertension	1 (1.5)	…	1 (1.4)
Septic shock	1 (1.5)	1 (1.5)	…
Chills	1 (1.5)	3 (4.4)	4 (5.8)
Tachycardia	1 (1.5)	3 (4.4)	…
Pyrexia	1 (1.5)	3 (4.4)	3 (4.4)
Renal failure/worsening	…	…	4 (5.8)
Drug reaction	…	…	3 (4.4)
Nausea	…	…	3 (4.4)
Abdominal pain	…	…	1 (1.4)
Diarrhea	…	1 (1.5)	…
Laboratory event			
Low monocyte count	1 (1.5)	1 (1.5)	…
Hypernatremia	…	…	1 (1.4)
Hypokalemia	…	4 (4.4)	5 (7.2)[a]
Hypocalcemia	…	…	1 (1.4)
Hyperlipidemia	…	1 (1.5)	…
Increase in urea and/or creatinine level	…	2 (2.9)	2 (2.9)
Anemia	…	1 (1.5)	2 (2.9)
Thrombocytopenia	…	1 (1.5)	…
Increase in liver enzyme level	…	2 (2.9)	3 (4.4)
Hematuria	…	…	1 (1.4)
Proteinuria	…	…	1 (1.4)
Total no. of events	11	27	39

NOTE. Data are no. (%) of patients. Mycograb (*Neu*Tec Pharma) is an antibody-based inhibitor of heat shock protein 90.

[a] An additional 4 patients developed hypokalemia, which was not attributed to amphotericin B use by the investigator.

DISCUSSION

To be of clinical benefit, a combination therapy regimen must improve efficacy without producing unacceptable toxicity. The regimen in the present study achieved this. The improved efficacy was evident from the increased frequency of clinical and mycological resolution at a fixed point (day 10; from 48% to 84%; $P < .001$), representing the minimum duration of L-amphotericin therapy, the reduced *Candida*-attributable mortality rate 4 weeks after completing study treatment (from 18% to 4%; $P = .025$), and culture-confirmed clearance of the infection occurring more than twice as quickly ($P = .001$). Resolution of infection in the placebo group was consistent with that obtained in clinical practice (30% of 923 adult patients and 37% of 174 pediatric patients, with candidiasis being cured by Abelcet, with an additional 30% of adults and 21% of pediatric patients showing improvement) [22, 27].

Likewise, patients with candidemia who received Mycograb were more likely to show an overall response (82% vs. 43%; $P = .002$), a clinical response (82% vs. 51%; $P = .01$), and a mycological response (88% vs. 51%; $P = .002$) at day 10. They also cleared their infections twice as quickly (HR, 2.4; $P = .011$) and had a reduced rate of *Candida*-attributable mortality (6% vs. 26%; $P = .04$).

Both mean APACHE II scores (17.5–18.0) and *Candida*-attributable mortality rates in the placebo arm were higher than in recent investigational studies, which have reported lower

APACHE II scores (mean scores, 13.8–15.4) and *Candida*-attributable mortality rates (4.4%–8.7%), probably reflecting more-exacting exclusion criteria and a higher proportion of *C. parapsilosis* infections (16%–20%) [2, 3]. In a large observational study ($n = 1447$) in which the mean APACHE II score (18.6) was higher than in these recent investigational studies, a higher rate of *Candida*-attributable mortality (14%) occurred among *C. albicans*–infected patients [4]. For *C. parapsilosis*, this rate was 2%. In studies that correlated early deaths (within 7 days of candidemia) with candidiasis, the mortality rates were 21% [28] and 22% [29].

Candidiasis was the most common cause of death in the placebo group, whereas the most common cause in the Mycograb group was multiple-organ failure secondary to bacterial sepsis. This was usually due to gram-negative bacterial infection and was often associated with complications of abdominal surgery. Recent or concomitant bacteremia, abdominal surgery, deep organ involvement, and multiple-organ failure all correlate with a poor prognosis in patients with candidiasis [22, 28–30].

Mycograb was well tolerated. Occasionally, patients experienced back pain, but the pain was transient, and neither the investigator nor the patient wished to discontinue therapy. Hypertension developed in 6% of patients within 2 h after receipt of the first dose of Mycograb; these patients had been receiving vasopressor support for sepsis-related hypotension at the time of study entry. Such patients should have their blood pressure monitored, particularly after receiving their first dose of Mycograb.

Hsp90 in normal cells is intracellular and released only on necrosis [31]. In certain cancers and yeasts, it is expressed on the cell surface, becoming a target for combination therapy, with hsp90 inhibition making the cell more susceptible to the second agent [14, 15, 32–34]. In cancer cells, antibody against hsp90 causes loss of invasiveness [35]. The liberation of hsp90 from yeast or necrotic human cells will activate endothelial nitric oxide synthase, leading to the release of nitric oxide—a vasodilator and a potent inhibitor of platelet aggregation [36, 37]. Heat-induced, hsp90-mediated peripheral vasodilatation in a human volunteer was reversed by hsp90 inhibition with geldanamycin [38]. Hsp90 activated the prekallikrein–high molecular weight kininogen complex on endothelial cells, resulting in bradykinin release [39]. This activity was reversed by an antibody against hsp90, and this system has been suggested as a counter-balance to the plasma rennin-angiotensin system [40]. Therefore, the cases of rebounded hypertension seen after receipt of the first dose of Mycograb could have been due to the rapid neutralization of high levels of circulating, vasodilatation-inducing hsp90.

Mycograb provides a new approach to the treatment of fungal infections, mimicking the host's natural protective antibody. Hsp90 inhibitors not only have direct antifungal activity, but they may also prevent fungi from developing resistance to fluconazole or caspofungin [14, 15]. Furthermore, the binding of Mycograb to circulating hsp90 may have additional clinical benefit in reversing hsp90-induced hypotension. These features make Mycograb a novel and unique antifungal.

STUDY GROUP MEMBERS

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