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Single-Dose Liposomal Amphotericin B Treatment for Cryptococcal Meningitis

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ABSTRACT

BACKGROUND

Cryptococcal meningitis is a leading cause of human immunodeficiency virus (HIV)–related death in sub-Saharan Africa. Whether a treatment regimen that includes a single high dose of liposomal amphotericin B would be efficacious is not known.

METHODS

In this phase 3 randomized, controlled, noninferiority trial conducted in five African countries, we assigned HIV-positive adults with cryptococcal meningitis in a 1:1 ratio to receive either a single high dose of liposomal amphotericin B (10 mg per kilogram of body weight) on day 1 plus 14 days of flucytosine (100 mg per kilogram per day) and fluconazole (1200 mg per day) or the current World Health Organization–recommended treatment, which includes amphotericin B deoxycholate (1 mg per kilogram per day) plus flucytosine (100 mg per kilogram per day) for 7 days, followed by fluconazole (1200 mg per day) for 7 days (control). The primary end point was death from any cause at 10 weeks; the trial was powered to show noninferiority at a 10-percentage-point margin.

RESULTS

A total of 844 participants underwent randomization; 814 were included in the intention-to-treat population. At 10 weeks, deaths were reported in 101 participants (24.8%; 95% confidence interval [CI], 20.7 to 29.3) in the liposomal amphotericin B group and 117 (28.7%; 95% CI, 24.4 to 33.4) in the control group (difference, –3.9 percentage points); the upper boundary of the one-sided 95% confidence interval was 1.2 percentage points (within the noninferiority margin; $P < 0.001$ for noninferiority). Fungal clearance from cerebrospinal fluid was $-0.40 \log_{10}$ colony-forming units (CFU) per milliliter per day in the liposomal amphotericin B group and $-0.42 \log_{10}$ CFU per milliliter per day in the control group. Fewer participants had grade 3 or 4 adverse events in the liposomal amphotericin B group than in the control group (50.0% vs. 62.3%).

CONCLUSIONS

Single-dose liposomal amphotericin B combined with flucytosine and fluconazole was noninferior to the WHO-recommended treatment for HIV-associated cryptococcal meningitis and was associated with fewer adverse events. (Funded by the European and Developing Countries Clinical Trials Partnership and others; Ambition ISRCTN number, ISRCTN72509687.)

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*A list of the Ambition Study Group members is provided in the Supplementary Appendix, available at NEJM.org.

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CRYPTOCOCCAL MENINGITIS IS THE MOST frequent cause of adult meningitis in areas with a high prevalence of human immunodeficiency virus (HIV)^{1,2} and is the second leading cause of HIV-related death worldwide, with the majority of deaths occurring in sub-Saharan Africa.³ Despite widened access to antiretroviral therapy, there is a persistent burden of advanced HIV disease in the sub-Saharan African region,⁴⁻⁶ and the number of cryptococcal meningitis cases remains high.^{6,7}

Poor outcomes with conventional antifungal treatment regimens are a key driver of the high mortality from cryptococcal meningitis, with a high incidence of toxic effects with the commonly used 2-week amphotericin B deoxycholate–based regimens and poor efficacy with fluconazole monotherapy, which has been associated with a 10-week mortality in excess of 50%.^{8,9} In 2018, after the publication of the Advancing Cryptococcal Meningitis Treatment for Africa (ACTA) trial,¹⁰ the World Health Organization (WHO) updated international guidelines to recommend induction therapy with the less toxic and more efficacious 1-week regimen of amphotericin B deoxycholate and flucytosine in resource-limited settings.¹¹ However, even 1 week of treatment with amphotericin B deoxycholate is associated with anemia, kidney impairment, and electrolyte abnormalities,⁸ and administering and monitoring intravenous amphotericin for 7 days poses logistic challenges in many clinical settings.

Liposomal amphotericin B is potentially well suited for use in short-course induction treatments of cryptococcal meningitis because it can be given at higher doses owing to a lower incidence of drug-induced toxic effects,¹²⁻¹⁴ has a long tissue half-life,^{12,13,15-17} and effectively penetrates into brain tissue.^{12,18,19} The concept of a single high-dose intravenous infusion of liposomal amphotericin B has been established in the treatment of visceral leishmaniasis,²⁰ and pharmacokinetic data from animal models and humans indicate that increasing the dose of liposomal amphotericin B from the currently recommended dose of 3 to 4 mg per kilogram of body weight may lead to improved outcomes in patients with cryptococcal meningitis and that short-course regimens may be as effective as daily therapy.^{15,16,21,22} In a phase 2 clinical trial, investigators assessed the efficacy of a short-course regimen with a single high dose of liposomal amphotericin B, two high doses of liposomal

amphotericin B given on days 1 and 3, or three high doses of liposomal amphotericin B given on days 1, 3, and 7, as compared with the control regimen of 14 daily doses of 3 mg per kilogram of liposomal amphotericin B (all four regimens included 14 days of high-dose fluconazole); they showed that the rate of fungal clearance from the cerebrospinal fluid with any of the three short-course, high-dose regimens was noninferior to that in the control group.²³ Maximal fungicidal activity was attained with a single 10-mg-per-kilogram dose of liposomal amphotericin B, and there was no evidence that additional doses led to greater benefit — findings that are in keeping with the data obtained from animal models.^{22,24} No safety concerns have been identified with the use of high-dose liposomal amphotericin B, which has a better adverse-effect profile than that observed with amphotericin B deoxycholate in previous trials.^{8,23}

On the basis of the findings of the phase 2 trial²³ and the data from a phase 3 trial that showed a role for flucytosine in the induction treatment of cryptococcal meningitis,¹⁰ we conducted an open-label, phase 3, randomized, controlled, noninferiority trial (the Ambition trial) to test a single high dose (10 mg per kilogram) of liposomal amphotericin B given with oral flucytosine and fluconazole for 2 weeks¹⁰ against the WHO-recommended first-line induction treatment with 1 week of amphotericin B deoxycholate plus flucytosine followed by 1 week of high-dose fluconazole.

METHODS

TRIAL DESIGN AND OVERSIGHT

The trial design has been described previously,²⁵ and the details are provided in the trial protocol, available with the full text of this article at NEJM.org. The protocol was approved by the London School of Hygiene and Tropical Medicine Research Ethics Committee and by the relevant ethics committees and national regulatory agencies overseeing the trial sites. All the participants provided written informed consent. If a participant had abnormal mental status, written informed consent was obtained from the next of kin; if a participant recovered the capacity to provide consent, written informed consent was obtained from that participant. An independent data-monitoring committee oversaw the trial and reviewed the trial data regularly. The trial funders,

suppliers, and drug manufacturers had no role in the design of the trial; in the collection, analysis, or interpretation of the data; or in the preparation of the manuscript or the decision to submit it for publication. Liposomal amphotericin B was donated by Gilead Sciences; amphotericin B deoxycholate was purchased from Bristol Myers Squibb; flucytosine was purchased from Mylan; and fluconazole was purchased from Cipla–Medopharm. At sites where the Pfizer Diflucan Partnership Program was operational, fluconazole donated by Pfizer was used if available. The authors vouch for the accuracy and completeness of the data and for the adherence of the trial to the protocol.

TRIAL PARTICIPANTS

HIV-positive adults (≥ 18 years of age) who had a first episode of cryptococcal meningitis, as diagnosed on the basis of a positive India ink stain or cryptococcal antigen test (CrAg lateral flow assay, IMMY) of a cerebrospinal fluid sample, were recruited from eight hospitals: Princess Marina Hospital, Gaborone, Botswana; Queen Elizabeth Central Hospital, Blantyre, and Kamuzu Central Hospital, Lilongwe, Malawi; Mitchells Plain Hospital and Khayelitsha Hospital, Cape Town, South Africa; Kiruddu National Referral Hospital, Kampala, and Mbarara Regional Referral Hospital, Mbarara, Uganda; and Parirenyatwa Central Hospital, Harare, Zimbabwe. Participants were excluded if they had received more than two doses of either amphotericin (at any dose) or fluconazole (at a dose of ≥ 800 mg) before screening; declined to consent or, if they had impaired capacity to consent, had no legal representative to consent on their behalf; were pregnant or breast-feeding; were taking contraindicated concomitant drugs; or had had any previous adverse reaction to a trial drug. Late-exclusion criteria, which were put in place to enable the rapid enrollment of critically ill participants pending baseline blood test results, were an alanine aminotransferase level greater than 5 times the upper limit of the normal range (>200 IU per liter), a polymorphonuclear leukocyte count of less than 500 per cubic millimeter, or a platelet count of less than 50,000 per cubic millimeter.

INTERVENTIONS AND RANDOMIZATION

Participants underwent randomization individually and were assigned in a 1:1 ratio to receive the experimental regimen that included a single

dose (10 mg per kilogram of body weight) of liposomal amphotericin B (AmBisome, Gilead Sciences) plus 14 days of flucytosine (100 mg per kilogram per day) and fluconazole (1200 mg per day)²⁶ or the current WHO-recommended regimen, which includes amphotericin B deoxycholate (1 mg per kilogram per day) plus flucytosine (100 mg per kilogram per day) for 7 days, followed by fluconazole (1200 mg per day) on days 8 through 14 (the control group). Randomization was performed with the use of a computer-generated randomization list with block sizes of four and six, stratified according to site. Randomization was performed electronically with a bespoke electronic data-capture tool in which the random-assignment sequence was concealed from all trial investigators involved in participant recruitment. The treatment-group assignments were provided to the recruiting teams after consent had been obtained and the participant enrolled. The trial medications were administered on an open-label basis.

All the participants were treated in-hospital for a minimum of 7 days. The single 10-mg-per-kilogram dose of liposomal amphotericin B was suspended in 1 liter of 5% dextrose and administered over the course of 2 hours, and the 1-mg-per-kilogram doses of amphotericin B deoxycholate were dissolved in 1 liter of 5% dextrose and administered over the course of 4 hours. Participants received 1 liter of intravenous normal saline before any amphotericin dose, plus at least 1 additional liter of intravenous fluid (5% dextrose or normal saline) on each day of amphotericin therapy. Potassium and magnesium supplements were given on each day that the participants received amphotericin and then for 2 additional days. Oral medications were administered through a nasogastric tube if participants were unable to swallow.

The results of laboratory blood tests were monitored regularly during the first 2 weeks and again at week 4. The monitoring schedule is provided in Table S1 in the Supplementary Appendix, available at NEJM.org. Lumbar punctures for quantitative cryptococcal cultures were performed at the time of diagnosis and on days 7 and 14. Participants with increased intracranial pressure received additional daily therapeutic lumbar punctures until the pressure was controlled at less than 20 cm of water.

Participants were followed at outpatient clinics for 10 weeks and were contacted by telephone

at week 16. If a participant missed a clinic appointment, follow-up was performed by the trial teams either by telephone or in person. After the 2-week induction period, all the participants received fluconazole at a dose of 800 mg per day for 8 weeks and then at a dose of 200 mg per day thereafter. Antiretroviral therapy was initiated, reinitiated, or switched to a new antiretroviral therapy with a different agent during weeks 4 to 6 and was chosen in accordance with national guidelines.

END POINTS

The primary end point was death from any cause at 10 weeks after randomization. As prespecified in the statistical analysis plan, the primary end point was tested for superiority after noninferiority was established. Secondary end points were death from any cause at 2 weeks, 4 weeks, and 16 weeks; overall mortality in a time-to-event analysis; the rate of fungal clearance from the cerebrospinal fluid per day over the course of 14 days of induction therapy; the percentage of participants in each trial group with clinical or laboratory-defined adverse events of grade 3 or 4, as determined according to the criteria of the Division of AIDS²⁷; and the median absolute or percentage change from baseline in laboratory values.

STATISTICAL ANALYSIS

Assuming 35% mortality at 10 weeks in both treatment groups, we calculated that a sample size of 390 per group (780 in total) would provide the trial with 90% power to show noninferiority of a single high dose of liposomal amphotericin B given with flucytosine and fluconazole to the current WHO recommended standard of care, with a specified noninferiority margin of 10 percentage points (the upper boundary of the one-sided 95% confidence interval of the absolute difference in mortality). The primary analysis was performed in the intention-to-treat population, which included all the participants who had undergone randomization and had not met any late-exclusion criteria. A generalized linear model with a binomial distribution was used to calculate the differences in mortality.

We performed two sensitivity analyses. First, a per-protocol analysis was performed in which participants were excluded if they had missed more than 1 day of any single treatment in the first 2 weeks or had missed more than 2 weeks

of fluconazole consolidation treatment between weeks 2 and 10. Second, we performed analyses that adjusted for the prespecified covariates of trial site, age, sex, baseline Glasgow Coma Scale score, CD4⁺ cell count, cryptococcal colony-forming units (CFU) per milliliter of cerebrospinal fluid, antiretroviral therapy status, hemoglobin level, and cerebrospinal fluid opening pressure. In the superiority, secondary end-point, and sensitivity analyses, no adjustments were made for multiple comparisons. Analysis of log-transformed longitudinal fungal counts in the cerebrospinal fluid was performed with the use of a linear mixed-effects model, in which undetectable measurements were left-censored (i.e., sterile cultures from day 7 onward were excluded if the values lessened the slope, because sterility would have been achieved before lumbar puncture on that day and use of these values would have therefore led to an underestimation of the true slope).²⁸ Adverse events were evaluated in the safety population, which included all the participants who had received one or more doses of a trial medication. Analyses were conducted with the use of SAS statistical software, version 9.4 (SAS Institute). The full statistical analysis plan is provided in the protocol.

RESULTS

TRIAL POPULATION

From January 2018 through February 2021, a total of 844 participants underwent randomization (Fig. 1). Of these participants, 30 were excluded — 24 met the prespecified late-exclusion criteria (13 had a low platelet count, 5 had a low neutrophil count, 2 had an increased alanine aminotransferase level, 3 had a low platelet count and a low neutrophil count, and 1 had a low platelet count and an increased alanine aminotransferase level [Table S2]), 5 did not have cryptococcal meningitis, and 1 was HIV-negative, which left 814 participants (407 in each treatment group) in the intention-to-treat population. None were lost to follow-up. An additional 30 participants were excluded from the per-protocol population (20 had missed more than 1 day of treatment in the first 2 weeks, 6 had received incorrect treatment, and 4 had missed more than 2 weeks of fluconazole consolidation treatment between weeks 2 and 10). The baseline characteristics of the participants were similar in the trial groups (Table 1).

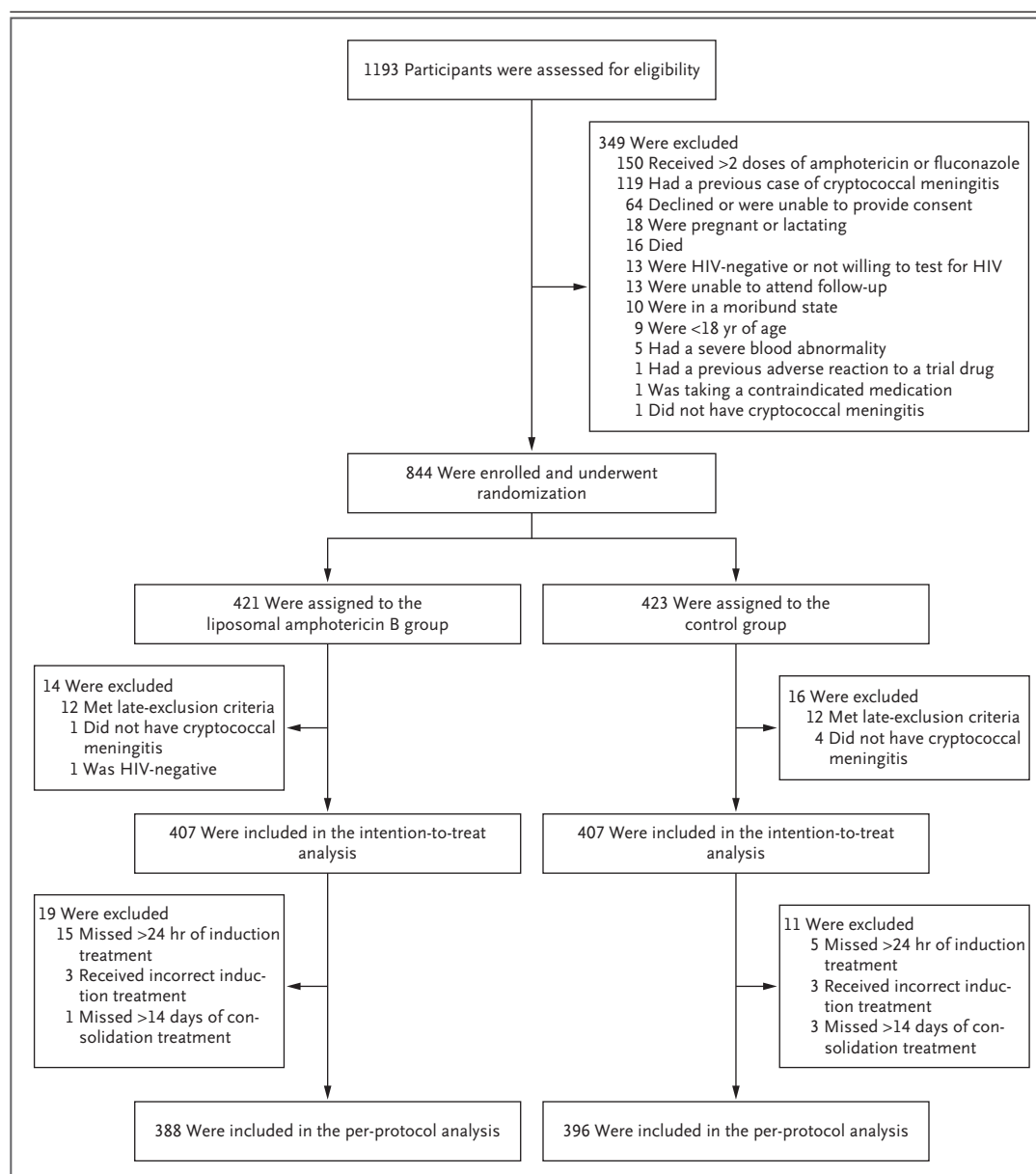


Figure 1. Screening, Randomization, and Analysis Populations.

The participants who were assigned to the liposomal amphotericin B group received a single dose of liposomal amphotericin B (10 mg per kilogram of body weight) plus 14 days of oral therapy with flucytosine (100 mg per kilogram per day) and fluconazole (1200 mg per day), and the participants assigned the control group received 7 days of amphotericin B deoxycholate (1 mg per kilogram) plus flucytosine (100 mg per kilogram per day) followed by 7 days of oral therapy with fluconazole (1200 mg per day). During the first week of induction therapy, two participants in the liposomal amphotericin B group received at least one dose of amphotericin B deoxycholate, and three participants in the control group received high-dose fluconazole. Participants may have had more than one reason for exclusion. HIV denotes human immunodeficiency virus.

PRIMARY END POINT

In the intention-to-treat analysis, 10-week mortality was 24.8% (95% confidence interval [CI], 20.7 to 29.3) in the liposomal amphotericin B group (101 of 407 participants had died) and

28.7% (95% CI, 24.4 to 33.4) in the control group (117 of 407 participants had died) (Table 2 and Fig. 2A). The absolute difference in mortality at 10 weeks between the liposomal amphotericin B group and control group was −3.9 percentage

Table 1. Baseline Characteristics of the Participants.*

Characteristic	Liposomal Amphotericin B (N = 407)	Control (N = 407)
Median age (IQR) — yr	37 (32–44)	37 (32–43)
Male sex — no. (%)	246 (60.4)	245 (60.2)
New diagnosis of HIV — no. (%)	127 (31.2)	118 (29.0)
Report of previous antiretroviral therapy — no. (%)†	256 (62.9)	266 (65.4)
Median weight (IQR) — kg	53 (47–60)	53 (48–60)
Headache		
Current symptom — no. (%)	390 (95.8)	394 (96.8)
Median duration (IQR) — days	14 (7–21)	14 (7–21)
Seizures within 72 hr before enrollment — no. (%)	45 (11.1)	42 (10.3)
Glasgow Coma Scale score <15 — no. (%)‡	115 (28.3)	117 (28.7)
Median values from CSF sample analysis (IQR)		
Cryptococcal quantitative value — CFU/ml	48,500 (300–420,000)	42,000 (585–365,000)
CSF opening pressure — cm of water	21 (14–32)	21 (13–31)
CSF opening pressure >25 cm of water — no./total no. (%)	165/399 (41.4)	158/400 (39.5)
White-cell count — cells/mm ³	6 (4–75)	5 (3–52)
Glucose level — mg/dl	45 (29–61)	43 (27–58)
Protein level — g/l	0.90 (0.46–1.48)	0.84 (0.44–1.38)
Median blood hemoglobin level (IQR) — g/dl	11.2 (9.7–12.7)	11.2 (9.6–12.9)
Median serum creatinine level (IQR) — mg/dl	0.7 (0.6–0.9)	0.8 (0.6–1.0)
Median blood CD4+ cell count (IQR) — cells/mm ³	26 (9–56)	28 (11–59)

* Baseline data were missing for the following characteristics: cerebrospinal fluid (CSF) cryptococcal quantitative value (missing for 1 participant in the liposomal amphotericin B group), CSF opening pressure (missing for 8 participants in the liposomal amphotericin B group and for 7 participants in the control group), CSF white-cell count (missing for 11 and 9 participants, respectively), CSF glucose level (missing for 11 and 15 participants, respectively), CSF protein level (missing for 14 and 16 participants, respectively), hemoglobin level (missing for 2 and 1 participant, respectively), CD4+ cell count (missing for 18 and 11 participants, respectively), and creatinine level (missing for 1 participant in the liposomal amphotericin B group). To convert the values for glucose to millimoles per liter, multiply by 0.05551. To convert the values for creatinine to micromoles per liter, multiply by 88.4. CFU denotes colony-forming units, HIV human immunodeficiency virus, and IQR interquartile range.

† The median interval from randomization to the reinitiation of antiretroviral therapy or switch to another therapy (for those with previous exposure to antiretroviral therapy) was 30 days in the liposomal amphotericin B group and 29 days in the control group.

‡ Scores on the Glasgow Coma Scale range from 3 to 15, with lower scores indicating worse mental status.

points, and the upper boundary of the one-sided 95% confidence interval was 1.2 percentage points, which was within the prespecified 10-percentage-point noninferiority margin ($P < 0.001$ for noninferiority) (Fig. 2B). In the per-protocol analysis, 10-week mortality at 10 weeks was 24.5% (95% CI, 20.3 to 29.1) in the liposomal amphotericin B group (95 of 388 participants had died) and 28.5% (95% CI, 24.1 to 33.3) in the control group (113 of 396 participants had died), for a between-group difference of –4.1 percentage points and an upper boundary of the

one-sided 95% confidence interval of 1.1 percentage points.

The results of the prespecified adjusted analyses (Table 2 and Fig. 2B) and key subgroup analyses (Table S3B) were consistent with those of the primary end-point analysis. In prespecified superiority analyses performed at the 10-week time point, the between-group difference in mortality was –3.9 percentage points with the 95% confidence interval crossing zero (95% CI, –10.0 to 2.2) in the unadjusted analysis and –5.7 percentage points with the 95% confidence in-

Table 2. Primary and Key Secondary End Points.*

Outcome	Unadjusted Analysis			Adjusted Analysis†		
	Liposomal Amphotericin B	Control	Difference (95% CI)‡	Upper Boundary of One-Sided 95% CI	Difference (95% CI)	Upper Boundary of One-Sided 95% CI
Death from any cause at 10 wk (primary end point)						
Intention-to-treat population						
Deaths — no./total no.	101/407	117/407				
Mortality (95% CI) — %	24.8 (20.7 to 29.3)	28.7 (24.4 to 33.4)	−3.93 (−10.0 to 2.2)	1.2§	−5.71 (−11.4 to −0.04)	−1.0
Per-protocol population						
Deaths — no./total no.	95/388	113/396				
Mortality (95% CI) — %	24.5 (20.3 to 29.1)	28.5 (24.1 to 33.3)	−4.05 (−10.2 to 2.1)	1.1	−5.04 (−10.8 to 0.8)	−0.2
Early fungicidal activity (key secondary end point)						
Participants with available data in the intention-to-treat population — no.¶	363	381				
Rate of fungal clearance over the course of 14 days — log ₁₀ CFU/ml/day						
Mixed-effects model	−0.40±0.13	−0.42±0.13	0.017 (−0.001 to 0.036)			
Linear-regression model	−0.41±0.19	−0.44±0.21	0.0270 (−0.004 to 0.058)			

* Plus-minus values are means ±SD.

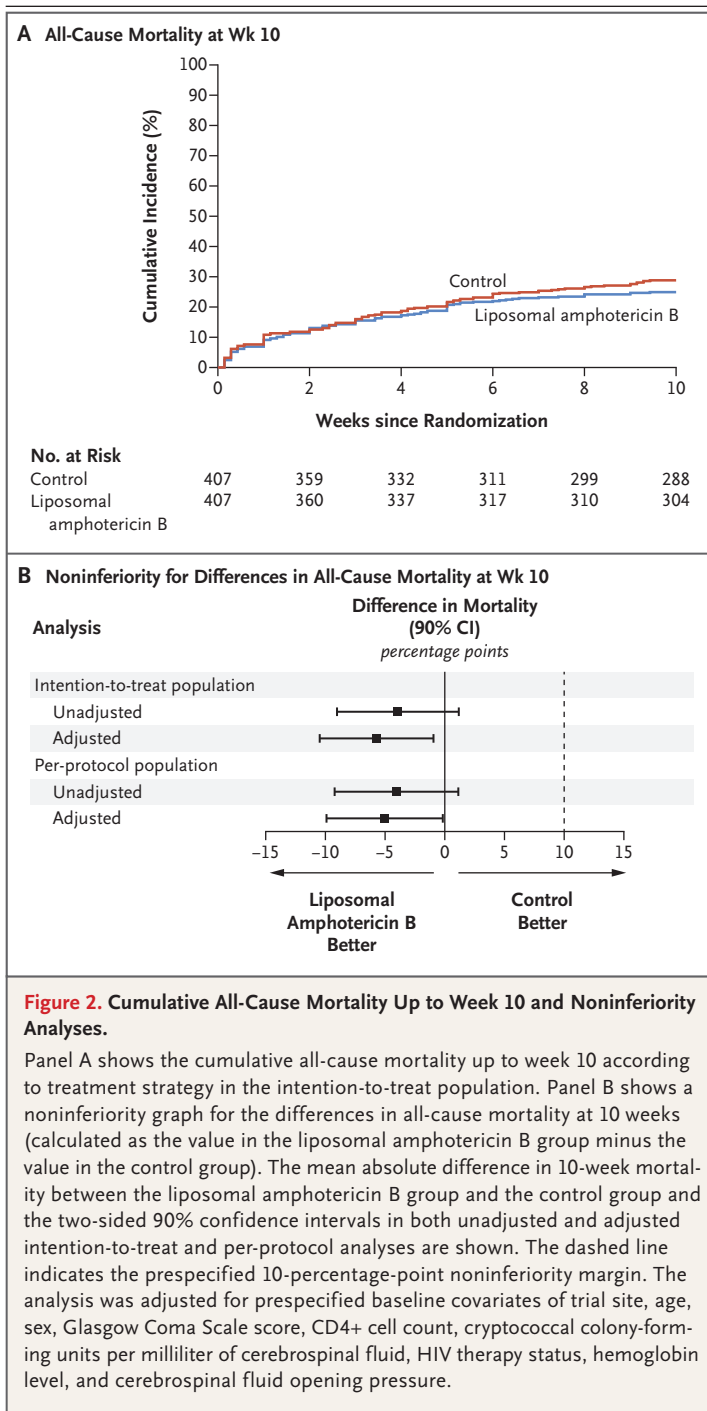
† The analysis was adjusted for the prespecified baseline covariates of site, age, sex, Glasgow Coma Scale score, CD4+ cell count, CSF cryptococcal quantitative culture, antiretroviral therapy status, hemoglobin level, and CSF opening pressure.

‡ The between-group difference is reported as the percentage-point difference for mortality and as absolute difference in the mean rate of fungal clearance for early fungicidal activity; the 95% confidence intervals are two-sided.

§ P<0.001 for noninferiority.

¶ Participants needed a nonsterile CSF culture at baseline to be included in this analysis.

|| To enable comparison with previously published data regarding early fungicidal activity that were derived from individual-patient linear-regression models, data were also analyzed by means of linear regression.



terval not crossing zero (95% CI, -11.4 to -0.04) in the analysis that was adjusted for the covariates associated with cryptococcal mortality.

SECONDARY END POINTS

Mortality at 2 weeks, 4 weeks, and 16 weeks is shown in Table S3. The results were consistent with the result of the primary end-point analysis

of 10-week mortality, with upper boundaries of the one-sided 95% confidence intervals of less than 10 percentage points. The results of time-to-event analyses of mortality that were performed with the use of Cox regression are shown in Table S4 and Figure 2A.

The mean rate of fungal clearance from the cerebrospinal fluid over the course of 14 days was $-0.40 \log_{10}$ CFU per milliliter per day in the liposomal amphotericin B group and $-0.42 \log_{10}$ CFU per milliliter per day in the control group, for a difference of $0.017 \log_{10}$ CFU per milliliter per day (95% CI, -0.001 to 0.036) (Table 2 and Fig. S2). Paradoxical immune reconstitution inflammatory syndrome was reported in 15 of 407 participants (3.7%) in the liposomal amphotericin B group and in 19 of 407 participants (4.7%) in the control group (Table S6). There were no cases of culture-positive relapse in the liposomal amphotericin B group. One case of relapse occurred in a participant in the control group who had received full induction therapy and had initial clearance of cryptococcus from the cerebrospinal fluid but subsequently had poor adherence to consolidation-phase fluconazole. During the initial 10 weeks of follow-up, 71 of 407 participants (17.4%) in each treatment group were re-admitted to the hospital at least once (Table S7).

SAFETY AND ADVERSE EVENTS

During the initial 21 days of treatment in the safety population, there were 382 grade 3 or 4 adverse events in 210 of 420 participants (50.0%) in the liposomal amphotericin B group and 579 grade 3 or 4 adverse events in 263 of 422 participants (62.3%) in the control group ($P < 0.001$). A summary of clinical and laboratory-defined adverse events is provided in Table 3, and a detailed list is provided in Table S8. Potentially life-threatening (grade 4) adverse events occurred in significantly fewer participants in the liposomal amphotericin B group than in the control group (91 of 420 participants [21.7%] vs. 127 of 422 participants [30.1%], $P = 0.005$). Grade 3 or 4 anemia developed in 56 of 420 participants (13.3%) in the liposomal amphotericin B group and in 165 of 422 participants (39.1%) in the control group ($P < 0.001$).

The mean decrease in hemoglobin level during the first week of the induction period was 0.3 g per deciliter in the liposomal amphotericin B group and 1.9 g per deciliter in the control group ($P < 0.001$); blood transfusion was performed

in 32 of 420 participants (7.6%) in the liposomal amphotericin B group and in 76 of 422 participants (18.0%) in the control group. A grade 3 or 4 increase in the creatinine level developed in 22 of 420 participants (5.2%) in the liposomal amphotericin B group and in 25 of 422 participants (5.9%) in the control group. The mean relative increase in the serum creatinine level from baseline to day 7 was 20.2% in the liposomal amphotericin B group and 49.7% in the control group ($P < 0.001$). Thrombophlebitis leading to antibiotic therapy occurred in 8 of 420 participants (1.9%) in the liposomal amphotericin B group and in 28 of 422 participants (6.6%) in the control group ($P = 0.001$). A low incidence of grade 4 thrombocytopenia, neutropenia, and elevated alanine aminotransferase level was observed in both treatment groups.

DISCUSSION

This trial showed that induction therapy with a single 10 mg-per-kilogram dose of liposomal amphotericin B in combination with oral flucytosine and fluconazole was noninferior to the WHO-recommended standard of care that included 1 week of amphotericin B deoxycholate given with flucytosine and was associated with significantly fewer adverse events. Because this clinical trial involving HIV-positive adults with cryptococcal meningitis was conducted in a range of health care settings across five countries in southern and eastern Africa with no loss to follow-up, our results are likely to be generalizable to other African settings with a high prevalence of HIV (Table S9).

The 10-week mortality of 24.8% observed in the liposomal amphotericin B group in our trial is among the lowest reported from a major cryptococcal meningitis trial in Africa, despite more than a quarter of participants presenting with very severe disease and abnormal baseline mental status. Our trial showed that either strategy (a single dose of liposomal amphotericin B plus 14 days of therapy with flucytosine and fluconazole or short-course treatment with 7 days of amphotericin B deoxycholate plus flucytosine followed by 7 days of fluconazole therapy) can reduce 10-week mortality from cryptococcal meningitis to below 30%. This finding represents a notable improvement on the rates of 40 to 45% reported in trials of 2-week amphotericin B deoxycholate-based regimens that were conducted

in resource-limited settings^{10,29-31} and is consistent with the relatively favorable outcomes with the 1-week regimen of amphotericin B deoxycholate plus flucytosine that were reported in the ACTA trial.¹⁰

Our trial builds on phase 2 data²³ showing that a single 10-mg-per-kilogram dose of liposomal amphotericin B is effective in clearing *Cryptococcus* from the cerebrospinal fluid. The effect on fungicidal activity with a single high dose of liposomal amphotericin B given with flucytosine and fluconazole matched that of 7 days of treatment with amphotericin B deoxycholate (1 mg per kilogram per day) plus flucytosine. In addition, the regimen that included a single high dose of liposomal amphotericin B led to fewer adverse effects than the 1-week amphotericin B deoxycholate regimen, with fewer adverse events overall, fewer life-threatening grade 4 events, fewer episodes of grade 3 or 4 anemia, a reduced need for blood transfusion, and less severe thrombophlebitis. These findings reflect the toxicity profile of liposomal amphotericin B that is known to be better than that of amphotericin B deoxycholate.^{12,14} In this trial, we administered preempive fluid and electrolytes to all the participants to reduce the risk of amphotericin B–related toxic effects, adopted an intensive blood-monitoring schedule, and actively managed adverse events when they occurred. The reality of routine care in resource-limited settings is that the necessary resources are often not available to implement measures to reduce toxic effects and an intensive monitoring and management approach.

An additional potential benefit of the liposomal amphotericin B regimen is that it may be possible to shorten the length of hospital stay needed to safely administer effective treatment. For the evaluation of safety in this trial, our protocol required that all participants be hospitalized for a 7-day period of inpatient monitoring. However, when scaled-up in real-world situations, earlier discharge will probably be possible for some patients. A cost-effectiveness comparison is under way. Given our results, a single high dose of liposomal amphotericin B may be worth investigating in the treatment of other systemic fungal infections that are prevalent in resource-limited settings, such as histoplasmosis and talaromycosis.^{32,33}

Our trial was open label, and clinical care of the critically ill participants with advanced HIV disease was complex. However, both the primary

Table 3. Laboratory-Defined and Clinical Adverse Events Occurring within 21 Days after Randomization.*

Event	Liposomal Amphotericin B (N=420)	Control (N=422)	P Value†
Grade 3 or 4 adverse events — no. of events	382	579	
Any grade 3 or 4 adverse event — no. of participants (%)			
Grade 3 or 4	210 (50.0)	263 (62.3)	<0.001
Grade 3	173 (41.2)	225 (53.3)	<0.001
Grade 4	91 (21.7)	127 (30.1)	0.005
Anemia — no. of participants (%)‡			
Grade 3	44 (10.5)	108 (25.6)	<0.001
Grade 4	12 (2.9)	62 (14.7)	<0.001
Mean change in hemoglobin level from baseline to day 7 — g/dl§	-0.3±1.39	-1.9±1.8	<0.001
Receipt of blood transfusion — no. of participants (%)	32 (7.6)	76 (18.0)	<0.001
Neutropenia — no. of participants (%)¶			
Grade 3	27 (6.4)	21 (5.0)	0.36
Grade 4	20 (4.8)	16 (3.8)	0.49
Thrombocytopenia — no. of participants (%)			
Grade 3	9 (2.1)	17 (4.0)	0.11
Grade 4	4 (1.0)	6 (1.4)	0.75
Creatinine increase — no. of participants (%)**			
Grade 3	17 (4.0)	22 (5.2)	0.42
Grade 4	5 (1.2)	3 (0.7)	0.51
Mean relative increase in creatinine level from baseline to day 7 — %††	20.2±48.1	49.7±70.8	<0.001
Hypokalemia — no. of participants (%)‡‡			
Grade 3	6 (1.4)	27 (6.4)	<0.001
Grade 4	0	3 (0.7)	0.25
Elevated ALT — no. of participants (%)§§			
Grade 3	6 (1.4)	4 (0.9)	0.52
Grade 4	1 (0.2)	1 (0.2)	1.0
Thrombophlebitis requiring antibiotic therapy — no. of participants (%)	8 (1.9)	28 (6.6)	<0.001
Other grade 3 or 4 adverse event — no. of participants (%)¶¶	167 (39.8)	173 (41.0)	0.72

* Plus-minus values are means ±SD. The adverse event data are presented for the safety population, which included all the participants who underwent randomization and received at least one dose of a trial medication. One participant in the liposomal amphotericin B group withdrew consent after randomization but before receiving any trial medication, and one participant in the control group died after randomization but before receiving any trial medication. Both participants were excluded from the safety analysis. ALT denotes alanine aminotransferase.

† P values were derived from chi-square or Student t-tests as appropriate.

‡ Grade 3 anemia was defined as a hemoglobin level of 7.0 to less than 9.0 g per deciliter in men and of 6.5 to less than 8.5 g per deciliter in women, and grade 4 as a hemoglobin level of less than 7.0 g per deciliter in men and of less than 6.5 g per deciliter in women.

§ Data regarding grade 3 events are reported for the participants who had both baseline and day 7 values available. Data were missing for 50 participants in the liposomal amphotericin B group and 61 in the control group.

¶ Grade 3 neutropenia was defined as a neutrophil count of 400 to 599 per cubic millimeter, and grade 4 as a neutrophil count of less than 400 per cubic millimeter.

|| Grade 3 thrombocytopenia was defined as a thrombocyte count of 25,000 to 49,999 per cubic millimeter, and grade 4 as a thrombocyte count of less than 25,000 per cubic millimeter.

** Grade 3 creatinine increase was defined as creatinine level of 2.47 to 4.42 mg per deciliter (216 to 400 μmol per liter), and grade 4 as a creatinine level of greater than 4.42 mg per deciliter.

†† Data regarding grade 4 events are reported for participants who had both baseline and day 7 values available. Data were missing for 42 participants in the liposomal amphotericin B group and 50 in the control group.

‡‡ Grade 3 hypokalemia was defined as a potassium level of 2.0 to 2.4 mmol per liter, and grade 4 as a potassium level greater than 2.4 mmol per liter.

§§ A grade 3 elevation in ALT level was defined as an ALT level of 200 to 400 IU per liter, and a grade 4 elevation as an ALT level greater than 400 IU per liter.

¶¶ During the course of the trial there were two infusion reactions that met the grade 3 criteria, both of which occurred in the liposomal amphotericin B group. Both cases responded to simple supportive measures. There were no participants in whom the prescribed dose of either liposomal amphotericin B or amphotericin B deoxycholate could not be given owing to infusion-related adverse events. We did not collate data on milder infusion reactions.

end point of death from any cause and the key safety end points of laboratory-confirmed toxic effects were objectively measured, and a consistent approach to HIV management and antiretroviral therapy was agreed on by the investigators and applied throughout the trial (Table S10) in order to avoid differential management strategies or outcome assessments in the treatment groups.

This trial showed that a single high dose of liposomal amphotericin B given with flucytosine and fluconazole was noninferior to the current WHO recommended standard of care for cryptococcal meningitis and offers a practical treatment for the management for HIV-associated cryptococcal meningitis that is easier to administer and associated with fewer drug-related adverse effects. Continued efforts to ensure access to liposomal amphotericin B and flucytosine are needed to enable the implementation of this treatment.

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APPENDIX

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REFERENCES

- Jarvis JN, Meintjes G, Williams A, Brown Y, Crede T, Harrison TS. Adult meningitis in a setting of high HIV and TB prevalence: findings from 4961 suspected cases. *BMC Infect Dis* 2010;10:67.
- Tenforde MW, Mokomane M, Leeme T, et al. Epidemiology of adult meningitis during antiretroviral therapy scale-up in southern Africa: results from the Botswana national meningitis survey. *J Infect* 2019;79:212-9.
- Rajasingham R, Smith RM, Park BJ, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *Lancet Infect Dis* 2017;17:873-81.
- Carmona S, Bor J, Nattey C, et al. Persistent high burden of advanced HIV disease among patients seeking care in South Africa's national HIV program: data from a nationwide laboratory cohort. *Clin Infect Dis* 2018;66:Suppl 2:S111-S117.
- Leeme TB, Mine M, Lechiile K, et al. Utility of CD4 count measurement in the era of universal antiretroviral therapy: an analysis of routine laboratory data in Botswana. *HIV Med* 2021;22:1-10.
- Osler M, Hilderbrand K, Goemaere E, et al. The continuing burden of advanced HIV disease over 10 years of increasing antiretroviral therapy coverage in South Africa. *Clin Infect Dis* 2018;66:Suppl 2: S118-S125.
- Tenforde MW, Mokomane M, Leeme T, et al. Advanced human immunodeficiency virus disease in Botswana following successful antiretroviral therapy roll-out: incidence of and temporal trends in cryptococcal meningitis. *Clin Infect Dis* 2017;65:779-86.
- Bicanic T, Bottomley C, Loyse A, et al. Toxicity of amphotericin B deoxycholate-based induction therapy in patients with HIV-associated cryptococcal meningitis. *Antimicrob Agents Chemother* 2015;59:7224-31.
- Tenforde MW, Gertz AM, Lawrence DS, et al. Mortality from HIV-associated meningitis in sub-Saharan Africa: a systematic review and meta-analysis. *J Int AIDS Soc* 2020;23(1):e25416.
- Molloy SE, Kanyama C, Heyderman RS, et al. Antifungal combinations for treatment of cryptococcal meningitis in Africa. *N Engl J Med* 2018;378:1004-17.
- Guidelines for the diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children. Geneva: World Health Organization, March 1, 2018 (<https://www.who.int/publications/i/item/9789241550277>).
- Adler-Moore J, Lewis RE, Brüggemann RJM, Rijnders BJA, Groll AH, Walsh TJ. Preclinical safety, tolerability, pharmacokinetics, pharmacodynamics, and antifungal activity of liposomal amphotericin B. *Clin Infect Dis* 2019;68:Suppl 4:S244-S259.
- Groll AH, Rijnders BJA, Walsh TJ, Adler-Moore J, Lewis RE, Brüggemann RJM. Clinical pharmacokinetics, pharmacodynamics, safety and efficacy of liposomal amphotericin B. *Clin Infect Dis* 2019;68:Suppl 4:S260-S274.
- Hamill RJ, Sobel JD, El-Sadr W, et al. Comparison of 2 doses of liposomal amphotericin B and conventional amphotericin B deoxycholate for treatment of AIDS-associated acute cryptococcal meningitis: a randomized, double-blind clinical trial of efficacy and safety. *Clin Infect Dis* 2010;51:225-32.
- Hope WW, Goodwin J, Felton TW, Ellis M, Stevens DA. Population pharmacokinetics of conventional and intermittent dosing of liposomal amphotericin B in adults: a first critical step for rational design of innovative regimens. *Antimicrob Agents Chemother* 2012;56:5303-8.
- O'Connor L, Livermore J, Sharp AD, et al. Pharmacodynamics of liposomal amphotericin B and flucytosine for cryptococcal meningoencephalitis: safe and effective regimens for immunocompromised patients. *J Infect Dis* 2013;208:351-61.
- Gubbins PO, Amsden JR, McConnell SA, Anaissie EJ. Pharmacokinetics and buccal mucosal concentrations of a 15 milligram per kilogram of body weight total dose of liposomal amphotericin B administered as a single dose (15 mg/kg), weekly dose (7.5 mg/kg), or daily dose (1 mg/kg) in peripheral stem cell transplant patients. *Antimicrob Agents Chemother* 2009;53:3664-74.
- Vogelsinger H, Weiler S, Djanani A, et al. Amphotericin B tissue distribution in autopsy material after treatment with liposomal amphotericin B and amphotericin B colloidal dispersion. *J Antimicrob Chemother* 2006;57:1153-60.
- Groll AH, Giri N, Petraitis V, et al. Comparative efficacy and distribution of lipid formulations of amphotericin B in experimental *Candida albicans* infection of the central nervous system. *J Infect Dis* 2000;182:274-82.
- Sundar S, Chakravarty J, Agarwal D, Rai M, Murray HW. Single-dose liposomal amphotericin B for visceral leishmaniasis in India. *N Engl J Med* 2010;362:504-12.
- Albert MM, Stahl-Carroll L, Luther ME, Graybill JR. Comparison of liposomal amphotericin B to amphotericin B for treatment of murine cryptococcal meningitis. *J Mycol Med* 1995;5:1-6.
- Lestner J, McEntee L, Johnson A, et al. Experimental models of short courses of liposomal amphotericin B for induction therapy for cryptococcal meningitis. *Antimicrob Agents Chemother* 2017;61(6): e00090-e17.
- Jarvis JN, Leeme TB, Molefi M, et al. Short-course high-dose liposomal amphotericin B for human immunodeficiency virus-associated cryptococcal meningitis: a phase 2 randomized controlled trial. *Clin Infect Dis* 2019;68:393-401.
- Lee JW, Amantea MA, Francis PA, et al. Pharmacokinetics and safety of a unilamellar liposomal formulation of amphotericin B (AmBisome) in rabbits. *Antimicrob Agents Chemother* 1994;38:713-8.
- Lawrence DS, Youssef N, Molloy SE, et al. AMBIsome Therapy Induction Optimisation (AMBITION): high dose AmBisome for cryptococcal meningitis induction therapy in sub-Saharan Africa: study protocol for a phase 3 randomised controlled non-inferiority trial. *Trials* 2018;19:649.
- Jackson AT, Nussbaum JC, Phulusa J, et al. A phase II randomized controlled trial adding oral flucytosine to high-dose fluconazole, with short-course amphotericin B, for cryptococcal meningitis. *AIDS* 2012;26:1363-70.
- Division of AIDS (DAIDS) table for grading the severity of adult and pediatric adverse events, corrected version 2.1. Bethesda, MD: U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS, July 2017 (<https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf>).
- Brouwer AE, Rajanuwong A, Chierakul W, et al. Combination antifungal therapies for HIV-associated cryptococcal meningitis: a randomised trial. *Lancet* 2004;363:1764-7.
- Beardsley J, Wolbers M, Kibengo FM, et al. Adjunctive dexamethasone in HIV-associated cryptococcal meningitis. *N Engl J Med* 2016;374:542-54.
- Day JN, Chau TTH, Wolbers M, et al. Combination antifungal therapy for cryptococcal meningitis. *N Engl J Med* 2013;368:1291-302.
- Boulware DR, Meza DB, Muzoora C, et al. Timing of antiretroviral therapy after diagnosis of cryptococcal meningitis. *N Engl J Med* 2014;370:2487-98.
- Johnson PC, Wheat LJ, Cloud GA, et al. Safety and efficacy of liposomal amphotericin B compared with conventional amphotericin B for induction therapy of histoplasmosis in patients with AIDS. *Ann Intern Med* 2002;137:105-9.
- Garcia A, Adler-Moore JP, Proffitt RT. Single-dose AmBisome (liposomal amphotericin B) as prophylaxis for murine systemic candidiasis and histoplasmosis. *Antimicrob Agents Chemother* 2000;44:2327-32.

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