ORIGINAL ARTICLE

Antifungal Combinations for Treatment of Cryptococcal Meningitis in Africa

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ABSTRACT

BACKGROUND

Cryptococcal meningitis accounts for more than 100,000 human immunodeficiency virus (HIV)–related deaths per year. We tested two treatment strategies that could be more sustainable in Africa than the standard of 2 weeks of amphotericin B plus flucytosine and more effective than the widely used fluconazole monotherapy.

METHODS

We randomly assigned HIV-infected adults with cryptococcal meningitis to receive an oral regimen (fluconazole [1200 mg per day] plus flucytosine [100 mg per kilogram of body weight per day] for 2 weeks), 1 week of amphotericin B (1 mg per kilogram per day), or 2 weeks of amphotericin B (1 mg per kilogram per day). Each patient assigned to receive amphotericin B was also randomly assigned to receive fluconazole or flucytosine as a partner drug. After induction treatment, all the patients received fluconazole consolidation therapy and were followed to 10 weeks.

RESULTS

A total of 721 patients underwent randomization. Mortality in the oral-regimen, 1-week amphotericin B, and 2-week amphotericin B groups was 18.2% (41 of 225), 21.9% (49 of 224), and 21.4% (49 of 229), respectively, at 2 weeks and was 35.1% (79 of 225), 36.2% (81 of 224), and 39.7% (91 of 229), respectively, at 10 weeks. The upper limit of the one-sided 97.5% confidence interval for the difference in 2-week mortality was 4.2 percentage points for the oral-regimen group versus the 2-week amphotericin B groups and 8.1 percentage points for the 1-week amphotericin B groups versus the 2-week amphotericin B groups, both of which were below the predefined 10-percentage-point noninferiority margin. As a partner drug with amphotericin B, flucytosine was superior to fluconazole (71 deaths [31.1%] vs. 101 deaths [45.0%]; hazard ratio for death at 10 weeks, 0.62; 95% confidence interval [CI], 0.45 to 0.84; P=0.002). One week of amphotericin B plus flucytosine was associated with the lowest 10-week mortality (24.2%; 95% CI, 16.2 to 32.1). Side effects, such as severe anemia, were more frequent with 2 weeks than with 1 week of amphotericin B or with the oral regimen.

CONCLUSIONS

One week of amphotericin B plus flucytosine and 2 weeks of fluconazole plus flucytosine were effective as induction therapy for cryptococcal meningitis in resource-limited settings. (ACTA Current Controlled Trials number, ISRCTN45035509.)

Cryptococcal meningitis is the most common form of adult meningitis in many regions that have a high prevalence of human immunodeficiency virus (HIV) infection[1,2] and accounts for 10 to 20% of all HIV-related deaths, with more than 100,000 deaths each year.[3] This high burden is driven by a high case fatality rate, which in sub-Saharan Africa is estimated to be 70% at 3 months.[3,4]

Treatment of cryptococcal meningitis in resource-limited settings is challenging. The international standard induction treatment of 2 weeks of amphotericin B deoxycholate plus flucytosine[5] is not available in most African clinical centers. Amphotericin B requires intravenous administration and close laboratory monitoring and is associated with phlebitis, secondary infections, anemia, and renal impairment.[6] Flucytosine is currently unavailable, although the molecule is used widely as a constituent of emtricitabine, and generic manufacture is possible at low cost.[7] Most countries therefore rely on generic or donated fluconazole induction monotherapy; however, the rate of fungal clearance with fluconazole is slower than that with amphotericin B, even at an elevated dosage, and mortality associated with this treatment is 50 to 60% at 10 weeks and is higher than 70% at 1 year even in study cohorts.[8,9]

Phase 2 studies have defined several promising treatment strategies that are associated with fungal clearance similar to that with 2-week amphotericin B regimens and that have more favorable safety profiles. An oral combination of fluconazole and flucytosine was found to be associated with a rate of clearance of infection similar to that with amphotericin B alone and to be associated with higher survival rates than those with fluconazole alone.[10] Shorter-course amphotericin B had a more favorable side-effect profile than standard 2-week courses, with no diminution in the rate of clearance of infection in the second week, perhaps because of the long half-life of amphotericin B in brain tissue.[11,12] The efficacy of shorter-course amphotericin B treatment has also been shown in animal models.[13]

In addition, the drug of choice to combine with amphotericin B remains unclear. In a previous trial, amphotericin B plus flucytosine was associated with higher survival rates at day 70 than amphotericin B alone.[14] Amphotericin B plus flucytosine was not found to differ from amphotericin B plus fluconazole with regard to mortality at 10 weeks, but the results of a secondary analysis at 6 months favored flucytosine.[14] However, these results have been insufficient to drive wider availability of flucytosine.[15]

Therefore, we tested two new treatment strategies that could be more readily sustainable in African centers than 2 weeks of amphotericin B and more effective than fluconazole: oral therapy with higher-dose fluconazole plus flucytosine, and a shorter course (1 week) of induction therapy with amphotericin B–based treatment. These regimens were compared with a 2-week regimen of amphotericin B–based treatment. In addition, within the amphotericin B groups, we randomly assigned patients to receive either flucytosine or fluconazole as the partner drug.

METHODS

TRIAL DESIGN AND OVERSIGHT

We conducted an open-label, phase 3, randomized, noninferiority, multicenter trial (Advancing Cryptococcal Meningitis Treatment for Africa [ACTA]) to compare three treatment strategies (an oral combination regimen of fluconazole plus flucytosine, 1 week of amphotericin B, and the standard 2 weeks of amphotericin B) for the induction treatment of HIV-associated cryptococcal meningitis. Flucytosine and fluconazole were also evaluated as partner drugs with amphotericin B.

Participants were recruited from nine African centers: Queen Elizabeth Central Hospital, Blantyre, Kamuzu Central Hospital, Lilongwe, and Zomba Central Hospital, Zomba, Malawi; University Teaching Hospital, Lusaka, Zambia; Muhimbili, Amana, and Mwananyamala Hospitals, Dar Es Salaam, Tanzania; and Hôpital Central, Yaoundé, and Douala General Hospital, Douala, Cameroon. The protocol was approved by the London School of Hygiene and Tropical Medicine Research Ethics Committee and by all the site national research ethics committees and regulatory bodies. Written informed consent was obtained from all the patients or, in the case of patients with altered mental status, from the next of kin (consent was obtained from these patients after recovery).

Lateral-flow cryptococcal antigen tests were donated by or purchased from IMMY. Trial drugs were purchased from Bristol-Myers Squibb (am-

photericin B [Fungizone]), Meda Pharmaceuticals (flucytosine), and Cipla or Medopharm (fluconazole). In places where the Pfizer fluconazole donation program was running, donated fluconazole was used when available. The trial funders, suppliers, and drug manufacturers had no role in trial design; data collection, analysis, and interpretation; or manuscript preparation. The authors vouch for the accuracy and completeness of the data and for the adherence of the trial to the protocol, available with the full text of this article at NEJM.org.

TRIAL PARTICIPANTS

HIV-seropositive adults (≥18 years old) with a first episode of cryptococcal meningitis who tested positive on India ink staining, cryptococcal antigen assay, or both in cerebrospinal fluid (CSF) were included. Patients were excluded if they had previously received more than one dose of amphotericin B or more than one treatment dose (1200 mg) or more than seven low doses (200 mg) of fluconazole in the 2 weeks before screening, were pregnant or lactating, were taking contraindicated concomitant drugs, or had any previous adverse reactions to the trial drugs.

An alanine aminotransferase (ALT) level that was more than 5 times the upper limit of the normal range, a polymorphonuclear leukocyte count that was less than 500 per cubic millimeter, or a platelet count that was less than 50,000 per cubic millimeter were late-exclusion criteria (i.e., a patient who met one or more of these criteria at baseline was withdrawn from the trial). In addition, if an elevated creatinine level remained above 220 μmol per liter on the day after randomization despite the patient receiving rehydration, the patient was withdrawn from the trial.

Initially, patients were excluded if they had previously been exposed to antiretroviral therapy (ART). However, because it became clear that a large number of patients were presenting with cryptococcal meningitis while taking ART or with previous exposure to ART, soon after commencement of the trial (after 4% of total enrollment), a protocol amendment allowed the inclusion of these patients. Full details of the trial design can be found in the protocol and statistical analysis plan.

INTERVENTIONS AND RANDOMIZATION

We assessed three treatment strategies (the use of an oral regimen, a 1-week amphotericin B regimen, and a 2-week amphotericin B regimen), as well as two alternative partner drugs for amphotericin B (fluconazole or flucytosine). The oral regimen consisted of fluconazole (1200 mg per day) plus flucytosine (100 mg per kilogram of body weight per day) given orally for 2 weeks. The 1-week amphotericin B regimen consisted of amphotericin B (1 mg per kilogram per day administered intravenously) plus either fluconazole (1200 mg per day) or flucytosine (100 mg per kilogram per day) for 7 days, followed on days 8 through 14 by fluconazole (1200 mg per day). The 2-week amphotericin B regimen consisted of amphotericin B (1 mg per kilogram per day administered intravenously) plus either fluconazole (1200 mg per day) or flucytosine (100 mg per kilogram per day) for 14 days.

Patients underwent block randomization individually, stratified according to site, to one of the three treatment strategies and, for patients who were assigned to an amphotericin B regimen, to one of the two partner drugs. Overall, this strategy resulted in a 2:1:1:1:1 ratio of patients assigned to receive one of the five combinations of treatment strategy and partner drug with amphotericin B. For each site, a computer-generated randomization list with block sizes of 18, 24, and 30 was produced. The trial pharmacist and clinician were responsible for conducting the randomization by sequentially drawing sealed envelopes that contained the treatment assignment for each enrolled patient.

Patients who received amphotericin B were given 1 liter of normal saline intravenously daily in addition to usual fluid requirements and preemptive potassium and magnesium (glycerophosphate) supplementation.[16] Oral medications were given through a nasogastric tube if the patient was unable to swallow. Laboratory blood tests were performed regularly during the first 2 weeks of treatment. Baseline and day 7 electrocardiographic monitoring was discontinued at the advice of the data and safety monitoring committee after 100 paired electrocardiograms showed no evidence of clinically significant prolongation of the QT interval in association with fluconazole at a dose of 1200 mg per day. Lumbar punctures were performed at baseline and on days 7 and 14 for quantitative cultures.[17] In addition, patients with high CSF pressure underwent daily therapeutic lumbar punctures until the pressure was controlled.[5] Patients were followed

for 10 weeks after randomization. After 2 weeks, fluconazole was given at 800 mg per day until ART was started at 4 weeks (or restarted in those who had discontinued ART), at 400 mg per day until 10 weeks, and at 200 mg per day thereafter. ART was prescribed in accordance with national guidelines.

END POINTS

The primary end point for comparison of the two experimental treatment strategies with the standard therapy of 2 weeks of amphotericin B–based treatment was all-cause mortality at 2 weeks. Two weeks was chosen in view of the noninferiority design of the trial and the fact that mortality at 2 weeks is more likely than mortality at later time points to reflect deaths from cryptococcal meningitis.[18] Secondary end points included 4-week and 10-week all-cause mortality, the rate of decrease in the $\log_{10}$ CSF fungal count over 14 days, and clinical and laboratory-defined grade 3 and 4 adverse events.

For the comparison between partner drugs for the amphotericin B regimens, the primary end point was all-cause mortality at 10 weeks. The secondary end points were all-cause mortality at 2 weeks and 4 weeks, rate of clearance of infection, and adverse events.

STATISTICAL ANALYSIS

A target enrollment of 680 patients (226 per strategy) was set in order to achieve 90% power to show noninferiority with a 10-percentage-point noninferiority margin and under the assumption of 15% mortality at 2 weeks in the 2-week amphotericin B groups. For the comparison of the partner drugs with amphotericin B, with the use of a superiority design and under the assumption of a 10-week mortality of 40% with one partner treatment, the trial had 90% power to detect a 35% lower mortality with the alternative partner treatment.

The primary analysis was based on the intention-to-treat population. A generalized linear model with a binomial distribution and identity link function was used to calculate differences and upper limits of the one-sided 95% confidence interval for mortality. Post-hoc analysis of the primary end point with a one-sided 97.5% confidence interval, the upper limit of which is equivalent to the upper limit of a two-sided 95% confidence interval and the use of which is equivalent to applying a Bonferroni correction ($\alpha = 0.025$) for two comparisons, was also performed. Correction for multiple comparisons was not applied to the analyses of secondary outcomes. The per-protocol population excluded patients who missed more than 1 day of treatment within the first 2 weeks after randomization.

All-cause mortality at 2, 4, and 10 weeks was compared between the groups with the use of log-rank tests. Kaplan–Meier plots were also constructed, and Cox regression models with treatment as a predictor were used to derive hazard ratios and two-sided 95% confidence intervals. Analyses were also performed with adjustment for prespecified covariates: site, age, sex, Glasgow Coma Scale score, CD4+ cell count, CSF fungal count at baseline, and ART status at baseline. Sensitivity analyses of all-cause mortality were performed under the assumption that all the patients who were lost to follow-up had died.

The analysis of the $\log_{10}$ CSF fungal count over a period of 14 days from baseline was performed with a linear mixed-effects model. For comparison with previous studies,[17-19] linear regression was also used to calculate slopes of the decrease in CSF fungal count for each patient, and the mean slopes were compared between the groups.

All analyses were performed with the use of SAS software, version 9.3 (SAS Institute). Additional details are provided in the Supplementary Appendix, available at NEJM.org.

RESULTS

TRIAL POPULATION

From January 2013 through November 2016, a total of 721 patients underwent randomization (Fig. 1). Of these patients, 43 were excluded from all analyses: 30 met predefined late-exclusion criteria, 3 immediately withdrew consent, 7 were negative for cryptococcal meningitis, and 3 had had cryptococcal meningitis previously. A total of 16 patients were excluded from the per-protocol analysis: 14 missed more than 1 day of treatment within the 2-week induction period, and 2 did not receive the correct randomly assigned treatment. Baseline characteristics were similar in the treatment groups and reflected the severity of immunosuppression in the population (Table 1, and Tables S1 and S2 in the Supplementary Ap-

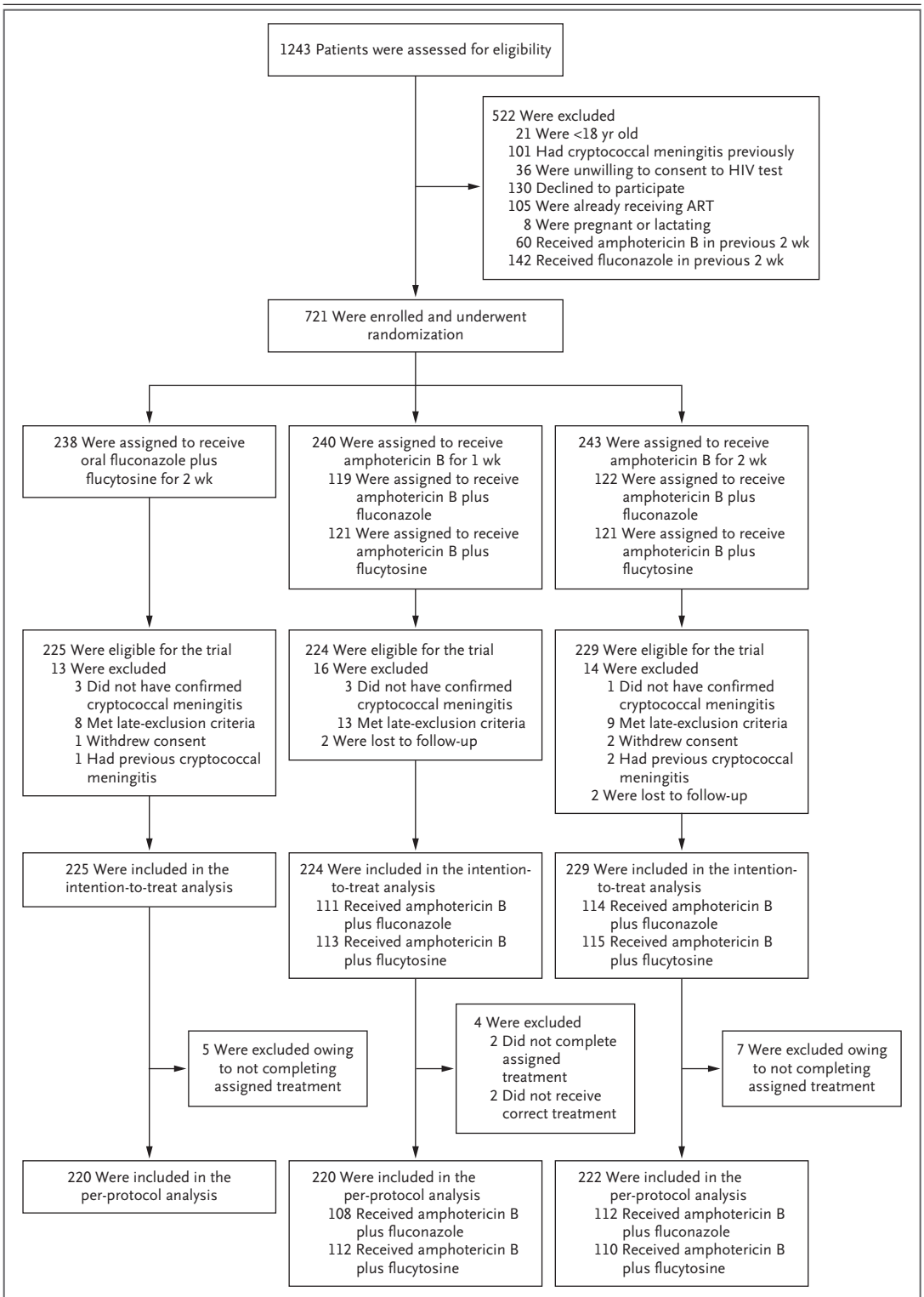

Figure 1. Screening, Randomization, and Analysis Populations.

Patients may have had more than one reason for exclusion. ART denotes antiretroviral therapy, and HIV human immunodeficiency virus.

Table 1. Baseline Characteristics of the Patients.*

Characteristic	Oral Regimen (N=225)	1-Wk Amphotericin B (N=224)	2-Wk Amphotericin B (N=229)
Male sex — no. (%)	119 (52.9)	137 (61.2)	134 (58.5)
Median age (IQR) — yr	36.0 (32.0–43.0)	38.5 (32.0–44.0)	37.0 (32.0–43.0)
Reported ART exposure — no. (%)†	128 (56.9)	119 (53.1)	134 (58.5)
Median weight (IQR) — kg‡	50 (46–60)	53 (47–60)	51 (46–60)
Current headache — no. (%)	221 (98.2)	221 (98.7)	226 (98.7)
Median duration of headache (IQR) — days§	14 (7–21)	14 (7–21)	14 (7–28)
Seizures within 72 hr before enrollment — no. (%)	40 (17.8)	43 (19.2)	36 (15.7)
Current fever — no. (%)	119 (52.9)	103 (46.0)	115 (50.2)
Current vision loss — no. (%)	17 (7.6)	17 (7.6)	22 (9.6)
Any cranial-nerve palsy — no. (%)	17 (7.6)	13 (5.8)	23 (10.0)
History of tuberculosis — no./total no. (%)	63/224 (28.0)	60/224 (26.8)	60/229 (26.2)
Glasgow Coma Scale score <15 — no. (%)¶	53 (23.6)	46 (20.5)	64 (27.9)
Abnormal mental status — no. (%)‖	101 (44.9)	90 (40.2)	107 (46.7)
Median CSF fungal count (IQR) — $\log_{10}$ CFU/ml**	5.0 (3.7–5.7)	5.0 (3.5–5.9)	5.0 (3.8–5.7)
Median CSF opening pressure (IQR) — cm††	22 (13–35)	24 (13–38)	25 (15–38)
CSF opening pressure >30 cm — no./total no. (%)††	69/218 (31.7)	78/211 (37.0)	80/215 (37.2)
Median CSF white-cell count (IQR) — cells/mm^3‡‡	4.0 (0.0–20.0)	4.0 (0.0–15.0)	3.0 (0.0–15.0)
Median CSF glucose level (IQR) — mmol/liter§§	2.0 (1.0–2.6)	2.0 (1.0–2.6)	2.0 (1.0–2.4)
Median CSF protein level (IQR) — mg/dl¶¶	113 (48–190)	102 (5–163)	99 (55–154)
Median hemoglobin level (IQR) — g/dl‖‖	10.7 (9.2–12.1)	11.0 (10.0–12.5)	10.9 (9.6–12.4)
Median creatinine level (IQR) — mg/dl***	0.7 (0.6–0.9)	0.8 (0.6–0.9)	0.7 (0.6–0.9)
Median baseline CD4+ cell count (IQR) — cells/mm^3†††	25 (10–63)	26.5 (12–63)	26 (10–64)

* ART denotes antiretroviral therapy, CFU colony-forming units, CSF cerebrospinal fluid, and IQR interquartile range.
† If only the patients who were enrolled after the ART amendment are considered, the proportions are 60.1% in the oral-regimen group, 55.6% in the 1-week amphotericin B groups, and 60.4% in the 2-week amphotericin B groups.
‡ Data were missing for 5 patients in the oral-regimen group, 6 in the 1-week amphotericin B groups, and 5 in the 2-week amphotericin B groups.
§ Data were missing for 4 patients in the oral-regimen group, 3 in the 1-week amphotericin B groups, and 3 in the 2-week amphotericin B groups.
¶ Scores on the Glasgow Coma Scale range from 3 to 15, with lower scores indicating lower levels of consciousness.
‖ Abnormal mental status was defined as having one or more of the following symptoms in the past 72 hours: drowsiness, behavioral change, or seizures.
** Data were missing for 10 patients in the oral-regimen group, 2 in the 1-week amphotericin B groups, and 8 in the 2-week amphotericin B groups. In total, 35 patients had CSF fungal burden of 0 at baseline: 9, 14, and 12 patients, respectively.
†† Data were missing for 7 patients in the oral-regimen group, 13 in the 1-week amphotericin B groups, and 14 in the 2-week amphotericin B groups.
‡‡ Data were missing for 4 patients in the oral-regimen group, 8 in the 1-week amphotericin B groups, and 10 in the 2-week amphotericin B groups.
§§ Data were missing for 27 patients in the oral-regimen group, 24 in the 1-week amphotericin B groups, and 26 in the 2-week amphotericin B groups.
¶¶ Data were missing for 24 patients in the oral-regimen group, 23 in the 1-week amphotericin B groups, and 32 in the 2-week amphotericin B groups.
‖‖ Data were missing for 1 patient in the oral-regimen group and 1 in the 2-week amphotericin B groups.
*** Data were missing for 4 patients in the oral-regimen group, 1 in the 1-week amphotericin B groups, and 1 in the 2-week amphotericin B groups. To convert values for creatinine to micromoles per liter, multiply by 88.4.
††† Data were missing for 16 patients in the oral-regimen group, 12 in the 1-week amphotericin B groups, and 18 in the 2-week amphotericin B groups.

Table 2. Unadjusted Analysis of Mortality and Rate of Fungal Clearance in CSF According to Treatment Strategy in the Intention-to-Treat Population.*

Outcome	Oral Regimen (N=225)	1-Wk Amphotericin B (N=224)	2-Wk Amphotericin B (N=229)	Difference (95% CI)†	
				Oral Regimen vs. 2-Wk Amphotericin B	1-Wk Amphotericin B vs. 2-Wk Amphotericin B
Mortality at 2 wk					
No. of deaths	41	49	49		
% (95% CI)	18.2 (13.2 to 23.3)	21.9 (16.5 to 27.4)	21.4 (16.1 to 26.7)	–3.18 (–10.50 to 4.15)	0.48 (–7.11 to 8.06)
Mortality at 4 wk					
No. of deaths	56	66	77		
% (95% CI)	24.9 (19.2 to 30.5)	29.5 (23.6 to 35.5)	33.6 (27.5 to 39.7)	–8.74 (–17.06 to –0.41)	–4.16 (–12.71 to 4.39)
Mortality at 10 wk					
No. of deaths	79	81	91		
% (95% CI)	35.1 (28.9 to 41.3)	36.2 (30.0 to 42.7)	39.7 (33.5 to 46.2)	–4.63 (–13.52 to 4.27)	–3.58 (–12.51 to 5.35)
Fungal clearance‡					
No. of patients	182	179	182		
Clearance rate — $\log_{10}$ CFU/ml/day	–0.26±0.18	–0.40±0.24	–0.42±0.25	0.10 (0.07 to 0.13)§	0.01 (–0.01 to 0.04)¶

* Plus–minus values are means ±SD. Patients who were lost to follow-up were included as alive in the analysis.
† Differences between mortality rates are given in percentage points. The upper limit of the two-sided 95% confidence interval is equivalent to that of the one-sided 97.5% confidence interval.
‡ Data are from a mixed-effects model with treatment, day, and interaction between treatment and day as fixed effects, the log baseline measurement of fungal count as a covariate, and patient as a random effect.
§ P<0.001 for the between-group difference.
¶ P=0.32 for the between-group difference.

pendix). In total, 59% of patients were taking or had previously taken ART. Patients who had never taken ART started the therapy at a median of 28 days (interquartile range, 27 to 34) after randomization.

MORTALITY

A total of 678 patients were eligible for inclusion in the intention-to-treat analyses. Of these patients, 1 was lost to follow-up within 2 weeks and 3 were lost to follow-up between 2 weeks and 10 weeks. Mortality was similar in the oral-regimen, 1-week amphotericin B, and 2-week amphotericin B groups: 18.2%, 21.9%, and 21.4%, respectively, at 2 weeks and 35.1%, 36.2%, and 39.7%, respectively, at 10 weeks (Table 2 and Fig. 2A). The upper limit of the one-sided 95% confidence interval for the difference in mortality at 2 weeks (primary end point) was 3.0 percentage points for the comparison of the oral-regimen group with the 2-week amphotericin B groups (P<0.001) and 6.8 percentage points for the comparison of the 1-week amphotericin B groups with the 2-week amphotericin B groups (P=0.007) (Fig. 2D). The upper limits of the one-sided 97.5% confidence intervals (the use of which is equivalent to applying a Bonferroni correction for the comparisons) both remained below the noninferiority margin (Table 2). The hazard ratios for death at 2 weeks, as compared with the 2-week amphotericin B groups, were 0.82 (95% confidence interval [CI], 0.54 to 1.25) in the oral-regimen group and 1.01 (95% CI, 0.68 to 1.51) in the 1-week amphotericin B groups; the corresponding hazard ratios for death at 10 weeks were 0.83 (95% CI, 0.61 to 1.13) and 0.89 (95% CI, 0.66 to 1.21) (Table S3 in the Supplementary Appendix). The results were similar in the per-protocol analysis, adjusted analysis, and sensitivity analyses (Fig. 2D, and Tables S4, S5, and S6 in the Supplementary Appendix).

As partner treatment with amphotericin B,

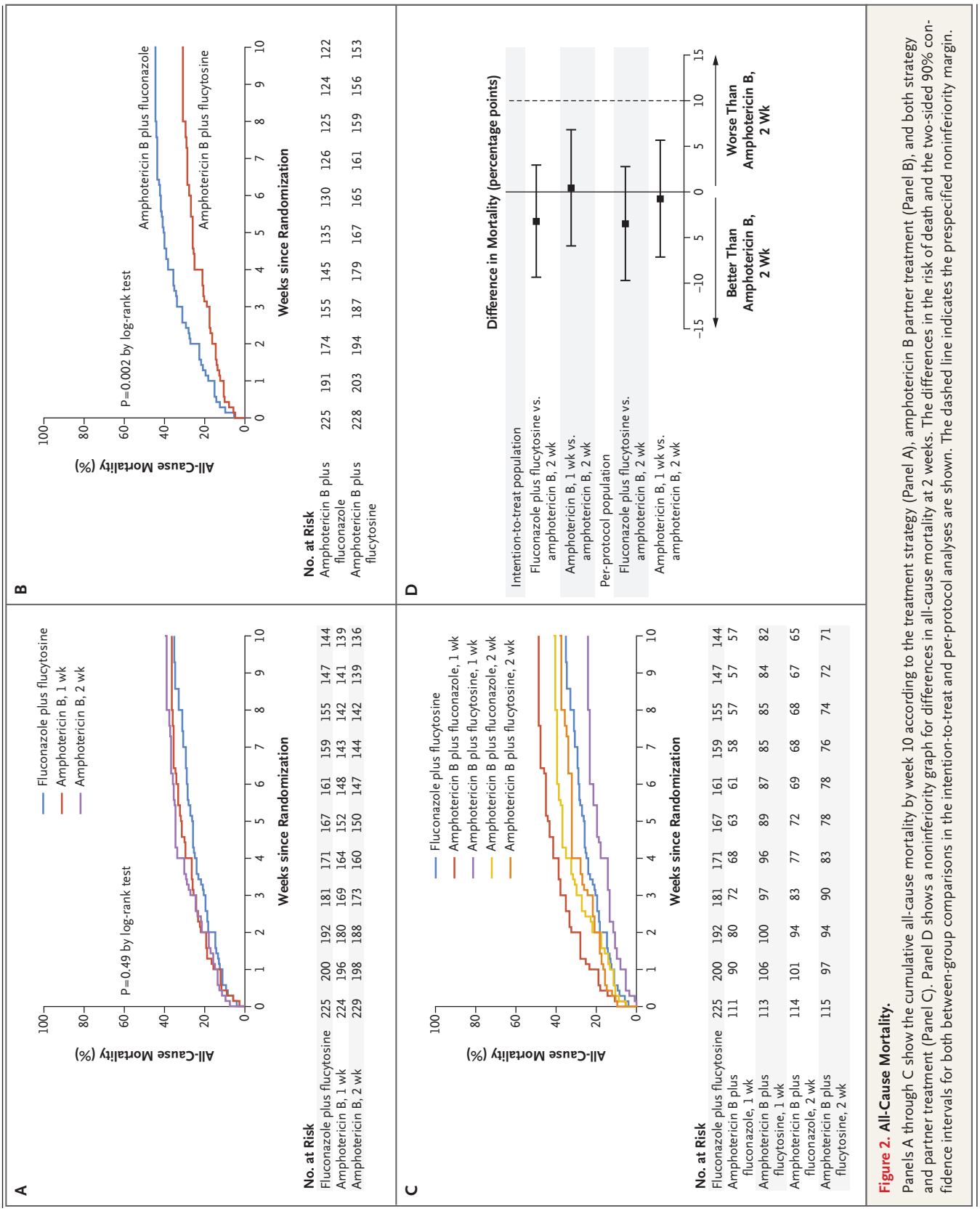

Figure 2. All-Cause Mortality.

Panels A through C show the cumulative all-cause mortality by week 10 according to the treatment strategy (Panel A), amphotericin B partner treatment (Panel B), and both strategy and partner treatment (Panel C). Panel D shows a noninferiority graph for differences in all-cause mortality at 2 weeks. The differences in the risk of death and the two-sided 90% confidence intervals for both between-group comparisons in the intention-to-treat and per-protocol analyses are shown. The dashed line indicates the prespecified noninferiority margin.

Table 3. Unadjusted Time-to-Event Analysis of Mortality and Rate of Fungal Clearance in CSF According to Partner Treatment with Amphotericin B in the Intention-to-Treat Population.*

Outcome	Amphotericin B + Fluconazole (N=225)	Amphotericin B + Flucytosine (N=228)	Hazard Ratio (95% CI)	P Value†
Mortality at 10 wk				
No. of deaths	101	71		
% (95% CI)	45.0 (38.5 to 51.5)	31.1 (25.3 to 37.3)	0.62 (0.45 to 0.84)	0.002
Mortality at 2 wk				
No. of deaths	61	37		
% (95% CI)	27.1 (21.3 to 32.9)	16.3 (11.5 to 21.1)	0.56 (0.37 to 0.85)	0.006
Mortality at 4 wk				
No. of deaths	86	57		
% (95% CI)	38.2 (31.9 to 44.6)	25.1 (19.4 to 30.7)	0.59 (0.42 to 0.83)	0.002
			Difference in Mean Clearance Rate (95% CI)	
Fungal clearance‡				
No. of patients	175	186		
Clearance rate — $\log_{10}$ CFU/ml/day	−0.36±0.23	−0.46±0.25	−0.06 (−0.03 to −0.08)	<0.001

* Plus–minus values are means ±SD. Missing values were not imputed.
† P values for the between-group differences in all-cause mortality were calculated with the use of a log-rank test.
‡ Data are from a mixed-effects model with treatment, day, and interaction between treatment and day as fixed effects, the log baseline measurement of fungal count as a covariate, and patient as a random effect.

flucytosine was superior to fluconazole (hazard ratio for death at 10 weeks with flucytosine vs. fluconazole, 0.62; 95% CI, 0.45 to 0.84; P=0.002) (Table 3 and Fig. 2B). This difference was driven by a difference in mortality between the 1-week amphotericin B–flucytosine group and the 1-week amphotericin B–fluconazole group. The results of separate analyses of the five groups are shown in Table 4 and Figure 2C, with the 2-week amphotericin B–flucytosine group as the comparator. The 1-week amphotericin B–flucytosine group had the lowest 10-week mortality (24.2%; 95% CI, 16.2 to 32.1), significantly lower than any other amphotericin B group (unadjusted hazard ratio, 0.56 [95% CI, 0.35 to 0.91], and adjusted hazard ratio, 0.59 [95% CI, 0.36 to 0.96], as compared with the 2-week amphotericin B–flucytosine group). The hazard ratio for death by 10 weeks, with the 1-week amphotericin B–flucytosine group used as the comparator, was 1.56 (95% CI, 1.01 to 2.42) in the oral-regimen group, 2.54 (95% CI, 1.60 to 4.05) in the 1-week amphotericin B–fluconazole group, and 1.97 (95% CI, 1.22 to 3.17) in the 2-week amphotericin B–fluconazole group (Table S7 in the Supplementary Appendix).

There was no significant difference in mortality between patients who had never taken ART and those who had previously been exposed to ART (Fig. S1 in the Supplementary Appendix).

RATE OF CLEARANCE OF INFECTION

The rate of clearance of infection (measured as the decrease in $\log_{10}$ colony-forming units per milliliter of CSF per day) was similar in the 1-week and 2-week amphotericin B groups and more rapid in the amphotericin B groups than in the oral-regimen group (Table 2). Flucytosine as the partner drug given with amphotericin B was associated with more rapid clearance than fluconazole (Table 3). Results were similar when linear regression was used, as in previous studies (Table S8 in the Supplementary Appendix).

SAFETY

Laboratory-defined side effects were less frequent in the oral-regimen group than in the 1-week or 2-week amphotericin B groups (Table 5, and Table S9 in the Supplementary Appendix). Grade 4 anemia developed in 0.9% of patients in the oral-regimen group, 4.9% of patients in the

Table 4. Unadjusted Time-to-Event Analysis of Mortality and Rate of Fungal Clearance in CSF According to Treatment Strategy and Partner Treatment with Amphotericin B in the Intention-to-Treat Population.*

Outcome	Oral Regimen (N=225)	1-Wk Amphotericin B + Fluconazole (N=111)	1-Wk Amphotericin B + Flucytosine (N=113)	2-Wk Amphotericin B + Fluconazole (N=114)	2-Wk Amphotericin B + Flucytosine (N=115)	Hazard Ratio vs. 2-Wk Amphotericin B + Flucytosine (95% CI)				P Value†
						Oral Regimen	1-Wk Amphotericin B + Fluconazole	1-Wk Amphotericin B + Flucytosine	2-Wk Amphotericin B + Fluconazole	
Mortality at 10 wk										
No. of deaths	79	54	27	47	44					
Mortality (95% CI) — %	35.1 (28.9 to 41.3)	48.6 (39.4 to 57.9)	24.2 (16.2 to 32.1)	41.3 (32.3 to 50.4)	38.3 (29.4 to 47.2)	0.87 (0.60 to 1.27)	1.42 (0.95 to 2.12)	0.56 (0.35 to 0.91)	1.10 (0.73 to 1.67)	0.001
Mortality at 2 wk										
No. of deaths	41	36	13	25	24					
Mortality (95% CI) — %	18.2 (13.2 to 23.3)	32.4 (23.7 to 41.1)	11.6 (5.7 to 17.5)	21.9 (14.3 to 29.5)	20.9 (13.4 to 28.3)	0.84 (0.50 to 1.39)	1.64 (0.97 to 2.78)	0.51 (0.26 to 1.00)	1.03 (0.59 to 1.82)	0.002
Mortality at 4 wk										
No. of deaths	56	46	20	40	37					
Mortality (95% CI) — %	24.9 (19.2 to 30.5)	41.4 (32.3 to 50.6)	17.8 (10.7 to 24.9)	35.1 (26.3 to 43.8)	32.2 (23.6 to 40.7)	0.74 (0.49 to 1.12)	1.41 (0.91 to 2.18)	0.50 (0.29 to 0.86)	1.10 (0.70 to 1.72)	<0.001
						Difference from 2 Wk Amphotericin B + Flucytosine in Mean Clearance Rate (95% CI)				
Fungal clearance‡										
No. of patients	182	81	98	94	88					
Clearance rate — $\log_{10}$ CFU/ml/day	−0.26±0.18	−0.36±0.23	−0.44±0.25	−0.37±0.24	−0.49±0.26	0.14 (0.11 to 0.17)§	0.08 (0.04 to 0.12)§	0.03 (−0.01 to 0.06)¶	0.06 (0.03 to 0.10)§	

* Plus–minus values are means ±SD. Missing values were not imputed.
† P values in this column pertain to the comparison of all five survival curves and were calculated with the use of the log-rank test.
‡ Data are from a mixed-effects model with treatment, day, and interaction between treatment and day as fixed effects, the log baseline measurement of fungal count as a covariate, and patient as a random effect.
§ P<0.001 for the difference from the 2-week amphotericin B–flucytosine group.
¶ P=0.16 for the difference from the 2-week amphotericin B–flucytosine group.

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1-week amphotericin B groups, and 8.8% of patients in the 2-week amphotericin B groups; the median decrease from baseline in hemoglobin level over the first 2 weeks was 0.4, 1.8, and 2.7 g per deciliter, respectively, and 5.5%, 10.3%, and 20.2%, respectively, of patients in each group received a transfusion. A grade 3 or 4 increase in the serum creatinine level developed in 4.9% of patients in the oral-regimen group, 6.2% of patients in the 1-week amphotericin B groups, and 8.8% of patients in the 2-week amphotericin B groups. Grade 4 hypokalemia developed in only one patient, most likely because preemptive electrolyte replacement was provided for patients receiving amphotericin B. Grade 4 neutropenia was recorded in 3.2% of the patients who were taking a regimen that included 2 weeks of flucytosine, in 0.9% of those taking 1 week of flucytosine, and in 1.3% of those taking a flucytosine-free regimen. A grade 4 increase in the alanine aminotransferase level developed in only two patients, one of whom was taking fluconazole. Clinical adverse events were frequent with all regimens, which was reflective of the severe immunosuppression in this patient population.

DISCUSSION

In this trial, we recruited patients from centers in southern, eastern, and central Africa, where the burden of cryptococcal meningitis is highest. We found that combination oral therapy with higher-dose fluconazole plus flucytosine and shorter-course 1-week amphotericin B–based treatment were noninferior to 2 weeks of amphotericin B–based therapy and that, as the partner drug with amphotericin B, flucytosine was associated with lower mortality than fluconazole. This latter difference was driven by the superiority of flucytosine to fluconazole in the 1-week amphotericin B groups. Indeed, although caution is appropriate in interpreting these results, given the secondary nature of the comparisons involved, 1 week of amphotericin B plus flucytosine was associated with higher survival rates than the other regimens, although the difference only just met the criteria for significance in the comparison with the oral combination and, in an adjusted analysis, in the comparison with the 2-week amphotericin B–flucytosine regimen.

The results were consistent in the per-protocol and intention-to-treat analyses, as well as in adjusted and sensitivity analyses. Few patients (0.6%) were lost to follow-up. Patients with severe disease were not excluded, so that the study population reflected patients presenting at centers across Africa. Also supportive of the generalizability of the results was the finding that mortality in the 2-week amphotericin B–fluconazole group (41.3% at 10 weeks) was the same as that seen in the placebo group of the recent multicenter trial of adjunctive glucocorticoids in which the same antifungal regimen was used.[20]

The results were consistent with those in animal models and in our phase 2 studies[10-13] and may reflect, at least in part, a balance between the rate of clearance of infection and the drug-related side effects. Flucytosine as the partner drug with amphotericin B was associated with more rapid clearance of infection than fluconazole and had a similar side-effect profile, as was previously shown in a study in Vietnam.[14] Clearance of infection was as rapid in the 1-week amphotericin B groups as it was in the 2-week amphotericin B groups, and, as expected, the shorter regimens had fewer side effects, with, in particular, less anemia. Our results with 1 week of amphotericin B–flucytosine lend further support to the concept of prolonged efficacy after an initial loading of brain compartments with amphotericin B,[13,21] as was also recently shown with the use of a single high dose of liposomal amphotericin B.[22] Of note, we implemented full preemptive management and monitoring of amphotericin B toxic effects.[16] It is likely that in resource-limited settings, the challenges of transfusion and monitoring would further disadvantage the 2-week amphotericin B regimens, as evidenced by the continued very high mortality rates reported from African centers where 2 weeks of amphotericin B has been used.[23,24] The amphotericin B–free oral combination regimen had few laboratory-defined side effects and also had efficacy, despite slower clearance of infection.

All the best-performing regimens in our trial contained flucytosine. In particular, mortality in the 1-week amphotericin B–flucytosine group was significantly lower than that in the other amphotericin B groups, whereas mortality in the 1-week amphotericin B–fluconazole group was the highest. It may be that the more effective partner drug is particularly important in the context of shorter courses of amphotericin B. In addition to rapid fungicidal activity, flucytosine may

Table 5. Laboratory-Defined and Clinical Adverse Events That Occurred within 21 Days after Randomization, According to Treatment Strategy.*

Event	Oral Regimen (N=225)	1-Wk Amphotericin B (N=224)	2-Wk Amphotericin B (N=228)
Any adverse event — no. of patients (%)			
Grade 3 or 4†	129 (57.3)	128 (57.1)	154 (67.5)
Grade 3	60 (26.7)	60 (26.8)	74 (32.5)
Grade 4	69 (30.7)	68 (30.4)	80 (35.1)
Anemia — no. of patients (%)			
Grade 3‡	9 (4.0)	20 (8.9)	40 (17.5)
Grade 4§	2 (0.9)	11 (4.9)	20 (8.8)
Median change in hemoglobin level to day 14 (IQR) — g/dl¶	–0.4 (–1.0 to 0.4)	–1.8 (–2.8 to –0.9)	–2.7 (–4.0 to –1.6)
Neutropenia — no. of patients (%)			
Grade 3‡	14 (6.2)	14 (6.2)	17 (7.5)
Grade 4§	8 (3.6)	3 (1.3)	4 (1.8)
Hypokalemia — no. of patients (%)			
Grade 3‡	3 (1.3)	14 (6.2)	15 (6.6)
Grade 4§	0	0	1 (0.4)
Thrombocytopenia — no. of patients (%)			
Grade 3‡	1 (0.4)	5 (2.2)	3 (1.3)
Grade 4§	4 (1.8)	2 (0.9)	1 (0.4)
Elevated ALT — no. of patients (%)			
Grade 3‡	6 (2.7)	6 (2.7)	7 (3.1)
Grade 4§	0	1 (0.4)	1 (0.4)
Creatinine increase — no. of patients (%)			
Grade 3‡	6 (2.7)	13 (5.8)	16 (7.0)
Grade 4§	5 (2.2)	1 (0.4)	4 (1.8)
Median change in creatinine level to day 14 (IQR) — μmol per liter‖	0 (–8.8 to 13.0)	14.0 (0.0 to 33.0)	35.4 (12.0 to 65.0)
Grade 3 or 4 pneumonia — no. of patients (%)	2 (0.9)	3 (1.3)	6 (2.6)
Grade 3 or 4 diarrhea or vomiting — no. of patients (%)	6 (2.7)	2 (0.9)	3 (1.3)
Grade 3 or 4 bacteremia or sepsis — no. of patients (%)	9 (4.0)	9 (4.0)	14 (6.1)
Other grade 3 or 4 adverse event — no. of patients (%)	120 (53.3)	125 (55.8)	148 (64.9)

* One patient who died after randomization but before receiving the trial treatment was excluded from the safety analysis.
† The total number of grade 3 or 4 adverse events (at any time after randomization) was 377 in the oral-regimen group, 465 in the 1-week amphotericin B groups, and 658 in the 2-week amphotericin B groups.
‡ The definitions of grade 3 adverse events were as follows: anemia, a hemoglobin level of 6.5 to 7.4 g per deciliter; neutropenia, a neutrophil count of 500 to 749 per cubic millimeter; hypokalemia, a potassium level of 2.0 to 2.4 mmol per liter; thrombocytopenia, a thrombocyte count of 25,000 to 49,999 per cubic millimeter; elevated alanine aminotransferase (ALT), an ALT level of 178 to 350 U per liter; and an increase in creatinine, a creatinine level of 2.47 to 4.42 mg per deciliter (218 to 390 μmol per liter).
§ The definitions of grade 4 adverse events were as follows: anemia, a hemoglobin level of less than 6.5 g per deciliter; neutropenia, a neutrophil count of less than 500 per cubic millimeter; hypokalemia, a potassium level of less than 2.0 mmol per liter; thrombocytopenia, a thrombocyte count of less than 25,000 per cubic millimeter; elevated ALT, an ALT level of more than 350 U per liter; and an increase in creatinine, a creatinine level of more than 4.55 mg per deciliter (402 μmol per liter).
¶ Data were missing for 35 patients in the oral-regimen group, 49 patients in the 1-week amphotericin B groups, and 46 patients in the 2-week amphotericin B groups.
‖ Data were missing for 34 patients in the oral-regimen group, 46 patients in the 1-week amphotericin B groups, and 39 patients in the 2-week amphotericin B groups.

also have other properties, such as a more prolonged postantibiotic effect than fluconazole,[25] to help explain this difference.

Despite the fact that patients in resource-limited settings have access to ART, the incidence of cryptococcal meningitis is not decreasing in many centers.[26,27] Widespread availability of generic flucytosine is urgently needed as an essential part of global programs to reduce HIV-related mortality, as is continued investigation into new drug therapies. Efforts by international agencies to make flucytosine available are gaining momentum.[7,15,28,29]

In conclusion, in our trial, 1 week of amphotericin B plus flucytosine was the most effective option for induction therapy for patients with HIV-associated cryptococcal meningitis in resource-limited settings. Our results also suggest that in the absence of availability of amphotericin B or in conditions in which amphotericin B cannot be administered safely, the oral combination of fluconazole plus flucytosine provides an effective and sustainable alternative.

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APPENDIX

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