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AmBisome Monotherapy and Combination AmBisome–Miltefosine Therapy for the Treatment of Visceral Leishmaniasis in Patients Coinfected With Human Immunodeficiency Virus in India: A Randomized Open-Label, Parallel-Arm, Phase 3 Trial

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Background. Visceral leishmaniasis (VL) in patients with human immunodeficiency virus (HIV) presents an increasingly important patient cohort in areas where both infections are endemic. Evidence for treatment is sparce, with no high-quality studies from the Indian subcontinent.

Methods. This is a randomized, open-label, parallel-arm, phase 3 trial conducted within a single hospital in Patna, India. One hundred and fifty patients aged ≥18 years with serologically confirmed HIV and parasitologically confirmed VL were randomly allocated to 1 of 2 treatment arms, either a total 40 mg/kg intravenous liposomal amphotericin B (AmBisome; Gilead Pharmaceuticals) administered in 8 equal doses over 24 days or a total 30 mg/kg intravenous AmBisome administered in 6 equal doses given concomitantly with a total 1.4 g oral miltefosine administered through 2 daily doses of 50 mg over 14 days. The primary outcome was intention-to-treat relapse-free survival at day 210, defined as absence of signs and symptoms of VL or, if symptomatic, negative parasitological investigations.

Results. Among 243 patients assessed for eligibility, 150 were recruited between 2 January 2017 and 5 April 2018, with no loss to follow-up. Relapse-free survival at day 210 was 85% (64/75; 95% CI, 77–100%) in the monotherapy arm, and 96%, (72/75; 90–100%) in the combination arm. Nineteen percent (28/150) were infected with concurrent tuberculosis, divided equally between arms. Excluding those with concurrent tuberculosis, relapse-free survival at day 210 was 90% (55/61; 82–100%) in the monotherapy and 97% (59/61; 91–100%) in the combination therapy arm. Serious adverse events were uncommon and similar in each arm.

Conclusions. Combination therapy appears to be safe, well tolerated, and effective, and halves treatment duration of current recommendations.

Clinical Trials Registration. Clinical Trial Registry India (CTRI/2015/05/005807; the protocol is available online at https://osf.io/avz7r).

Keywords. visceral leishmaniasis; liposomal amphotericin B; HIV; miltefosine; India.

One-third of all patients with human immunodeficiency virus (PHIV) worldwide live in regions where leishmaniasis is endemic [1]. Visceral leishmaniasis (VL) caused by the parasite *Leishmania donovani* is endemic to Bihar, a populous state of 125 million people in North India, which has, until very recently, carried an estimated 40% of the global VL burden [2]. Moreover, Bihar is one of the few states in India where the rate of new HIV infections is increasing [3]. This has major implications for VL–HIV coinfection: like other opportunistic infections in PHIV, *Leishmania* amastigotes have evolved strategies to survive [4], which are enhanced by HIV coinfection [5] and accelerate progression of disease [6]. This may help explain why the risk of developing VL is estimated to be between 100 and 2300 times higher in individuals with HIV than in those who are HIV negative [7].

Evidence of treatment for coinfected patients is limited due to a lack of randomized trials and is mostly from observational studies with relatively short follow-up periods, and often with high rates of loss to follow-up [8]. Nevertheless, worse outcomes

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in almost every respect have consistently been reported in this patient group when compared with patients not known to be HIV positive.

More recently, evidence from a noncomparative randomized trial of 59 VL–HIV coinfected patients in Ethiopia has been published examining a combination of AmBisome (liposomal amphotericin B; total dose of 30 mg/kg in 6 divided doses) with miltefosine (100 mg/day for 28 days) and AmBisome monotherapy (total dose of 40 mg/kg in 8 divided doses over 24 days). This showed poor performance in the monotherapy arm (adjusted efficacy at day 58 of 55%; 95% confidence interval [CI], 32–78%), whereas the combination arm reached an 88% (95% CI, 79–98%) efficacy rate at the end of treatment, albeit an extended repeat treatment strategy was required for substantial numbers of initial treatment failures in both arms. Furthermore, the addition of a secondary prophylaxis due to high relapse rates seen in the geographical context limited evaluation of long-term primary treatment efficacy [9].

Considering the very different regional behavior of the host–parasite relationship [10], it appears evident that results from 1 region and/or species of *Leishmania* cannot be extrapolated to others. We therefore evaluated the efficacy at 6 months and safety of a combination of intravenous AmBisome (total dose of 30 mg/kg in 6 divided doses) given concurrently with oral miltefosine (total dose of 1.4 g over 14 days in 2 daily 50-mg doses) and intravenous AmBisome monotherapy (total dose of 40 mg/kg in 8 divided doses) in Bihar, India.

METHODS

Study Design

We designed a parallel-arm, open-label, randomized, noncomparative phase 3 trial to investigate the safety and efficacy of 2 treatment regimens for VL in patients coinfected with HIV in Bihar, India. The study was conducted in a specialist VL research hospital in Patna, the state capital of Bihar. A trial with a superiority framework, to detect a relatively small difference, would have required a sample size that was not considered feasible to complete the study within a reasonable time frame. This also considered that powering for noninferiority would substantially increase the sample size, while the lack of evidence for the monotherapy arm would prevent meaningful noninferiority margins to be set. Hence, we opted for a noncomparative framework in which each arm was powered to detect a benefit of its regimen over a fixed value of the proportion cured.

Ethical approval was obtained from the Rajendra Memorial Research Institute of Medical Sciences Ethics Committee, the Médecins Sans Frontières Ethics Review Board, and the Research Ethics Committee of the London School of Hygiene and Tropical Medicine. The study was overseen by an independent data safety and monitoring board (DSMB). The study was prospectively registered at the Clinical Trial Registry India as CTRI/2015/05/005807 and the protocol is available online at https://osf.io/avz7r. All patients gave written consent.

Participants

All patients aged 18 years or older with a confirmed diagnosis of HIV (as per Indian National AIDS Control Organisation guidelines) with a parasitologically confirmed diagnosis of VL (bone marrow or spleen aspirate), regardless of previous episodes of VL, met the inclusion criteria for the study. Patients were screened at admission for tuberculosis (TB) using clinical examination and sputum cartridge-based nucleic acid amplification test (CBNAAT).

Patients were excluded if they demonstrated clinical or biological evidence of severe serious underlying disease that would preclude evaluation of participant response to the study medication. Women of child-bearing potential who were not using or unwilling to use an assured method of contraception were excluded due to miltefosine teratogenicity, as were pregnant and lactating women. Patients with hypersensitivity to the study drugs and those with a baseline serum creatinine of more than 1.2 mg/dL were also excluded.

Randomization

Subjects were allocated to treatment arm using block randomization, using random block sizes of 4, 6, and 8. The random number seed was set by an independent statistician who then ran the program. To achieve allocation concealment, randomization codes were placed in sealed, sequentially numbered, opaque envelopes by an independent person who had no further involvement in the rest of the trial. Patients and treating physicians were not blinded to the study treatment due to the predictable differences in dosing modes and schedules of investigational drugs.

Procedures

Patients received inpatient care for a minimum of 29 days at the Rajendra Memorial Research Institute of Medical Sciences Patna, a specialist regional research center for VL. Patients allocated to the liposomal amphotericin B (AmBisome; Gilead Pharmaceuticals, Foster City, CA) monotherapy arm were given a total dose of 40 mg/kg administered by slow intravenous (IV) infusion of 5 mg/kg on days 1–4, 8, 10, 17, and 24, as adapted from current World Health Organization (WHO) treatment recommendations [11]. Those allocated to the combination therapy arm were given a total dose of 30 mg/kg AmBisome by IV slow infusion of 5 mg/kg on days 1, 3, 5, 7, 9, and 11 concomitantly with a total dose of 1.4 g miltefosine (Impavido; Paladin Therapeutics, Inc, Canada) administered as one 50-mg capsule orally twice a day for 14 days.

During treatment, patients were assessed as inpatients on days 1, 3, 10, and 29, with parasitological test of cure conducted on day 29. Following discharge, patients were assessed on day 58 (±10 days), day 210 (±1 month), and day 390 (±2 months),

including measurement of clinical, hematological, and biochemical parameters. Where patients were suspected of relapse, repeat parasitological testing was done. Patients were also monitored for post–kala-azar dermal leishmaniasis.

Antiretroviral therapy (ART) was continued in patients already established on treatment unless there was a clinical indication otherwise; those with newly diagnosed HIV were initiated on ART on day 15 unless clinically contraindicated. Oral cotrimoxazole preventive therapy was given as per national guidelines.

Outcomes

To evaluate the safety and efficacy of both treatment regimens, we set the primary endpoint as relapse-free survival at 6 months (day 210) after the start of treatment, defined as being alive with an absence of signs and symptoms of VL or, if symptomatic, a negative parasitological assessment by tissue aspirate (spleen or bone marrow). The secondary endpoints were set as follows: (1) initial cure at day 29 post start of treatment, defined as cessation of fever, reduction in any initial splenomegaly, and a negative parasitological assessment; (2) relapse-free survival at 12 months (day 390); and (3) relapse-free survival at day 210 and 390 in the subset of patients with no diagnosis of TB coinfection prior to day 58.

Assessment of safety during treatment and follow-up was based on clinical adverse events and laboratory parameters recorded during the period of treatment and 1-month follow-up period (ie, up to day 58). Safety assessments were performed at baseline and days 3, 10, 29, and 58, and grading of event severity was based on the Common Terminology Criteria for Adverse Events (CTCAE), version 4.0. CD4 counts were measured at baseline and days 29, 210, and 390, while HIV viral load was measured at baseline and days 210 and 390.

Statistical Analysis

As the trial was noncomparative, the primary endpoint (relapse-free survival at day 210) was analyzed as a proportion for each arm (p). Confidence intervals for this endpoint were calculated using the score (Wilson) method [12]. As the hypothesis was 1-sided with significance level of 5%, a 1-sided 95% CI—p greater than the lower confidence limit—was presented as the primary analysis. A 2-sided 90% CI was also calculated. All secondary endpoints were analyzed in the same way as the primary endpoint. A paired t test compared CD4 counts over time within each arm.

The sample size of each arm was originally calculated as 56 patients to give 80% power to show a primary endpoint greater than 80%, assuming the true value of the endpoint is at least 90% [13]. However, after the recruitment of two-thirds of the patients, it became clear that an unexpectedly large number (19%, n = 28) were coinfected with TB in addition to VL-HIV. This high number became apparent through CBNAAT screening of all patients, something included for the first time in studies of VL-HIV coinfection. The potential to interfere with the interpretation of results was raised by the DSMB, since all 3 infections have different clinical characteristics and VL-HIV-TB coinfection is relatively unknown [14]. The DSMB recommended increasing the sample size per arm from 56 to 75, so that the non-TB subset would number approximately the same as originally planned for each arm (ie, 56, which is 75% of 75), allowing room in case the final proportion of TB cases was slightly higher. A subgroup analysis restricted to those without TB at time of enrollment (defined by the DSMB as diagnosed on or before day 58) was added as an additional secondary objective.

For all endpoint analysis, both an intention-to-treat (ITT) and per-protocol (PP) analysis were planned. In addition, the cumulative incidence of relapse was recalculated to allow for the presence of competing risks (death or treatment failure precluding the occurrence of relapse), since, in this case, standard survival methods can lead to biased estimates [15].

Data analysis was performed using Stata statistical software (release 15; StataCorp, College Station, TX). All adverse events were coded according to the *Medical Dictionary for Regulatory Activities* (MedDRA, version 23.1).

RESULTS

A total of 243 patients were assessed for eligibility between 2 January 2017 and 5 April 2018, of whom 42 (17%) had negative parasitology and 51 (21%) did not meet the inclusion criteria or met the exclusion criteria, leaving 150 (62%) enrolled (Figure 1) randomized equally between study arms. All participants were assessed in the ITT for the primary (and secondary) endpoints. No participants were lost to follow-up, and no primary endpoint data were missing. A PP analysis excluding 2 patients (1 patient missing the final AmBisome dose, and 1 patient with AmBisome hypersensitivity switched to rescue treatment) is included as Supplementary Material 1. Distributions of baseline demographic and clinical characteristics were similar in both groups (Table 1), including body mass index, CD4 count, viral load, and prior antiretroviral treatment (Supplementary Material 5); however, there was a higher proportion of baseline relapses in the monotherapy arm (53% vs 39%). Nineteen percent (n = 28) of enrolled participants overall were diagnosed with TB prior to day 58; these participants were distributed equally between study arms. Those participants (n = 3) diagnosed with TB after day 58 (on days 87, 227, and 241) were classified as non–TB infected.

The majority of participants had low baseline CD4 counts, which appeared unrelated to ART status in both groups (Supplementary Materials 2 and 3), with only 14% (n = 21) of all participants having CD4 counts greater than 200 cells/μL. In contrast, 72% (n = 47/65) of participants on ART at baseline showed HIV viral load suppression (<1000 copies/mL).

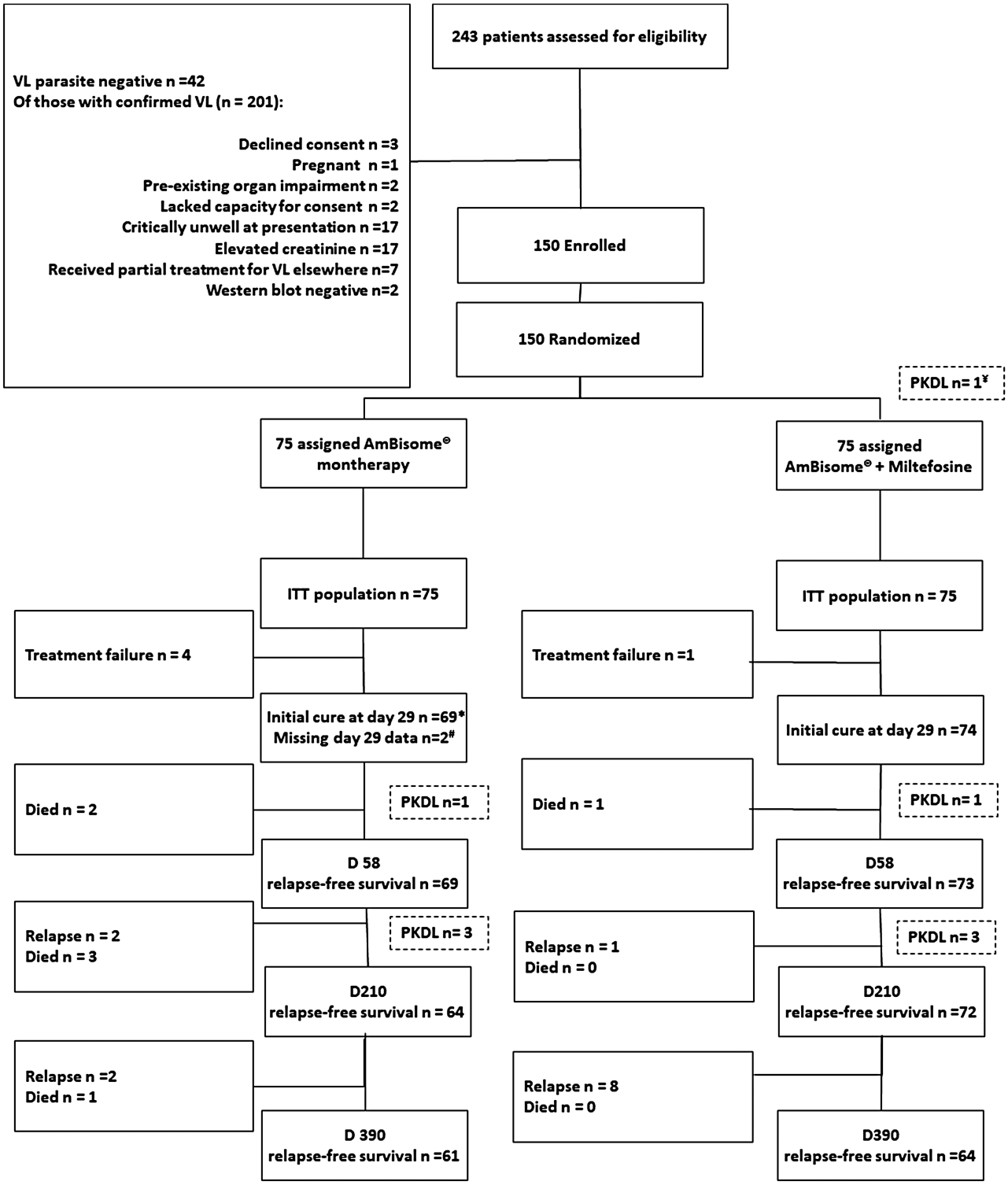


Figure 1. Trial profile. Abbreviations: D, day; ITT, intention-to-treat; PKDL, post–kala-azar dermal leishmaniasis; VL, visceral leishmaniasis.

In the monotherapy arm, 85% (n = 64) (95% CI, 77–100%) met the primary efficacy endpoint of relapse-free survival at 6 months. In the combination therapy arm, this was 96% (n = 72; 95% CI, 90–100%). Mortality in the monotherapy arm by day 210 was 6.7% (n = 5) and was 1.3% (n = 1) in the combination arm (Table 2), with 1 further death occurring after day 210 in

Table 1. Baseline Participant Characteristics

Characteristics [Normal Range]	AmBisome	AmBisome + Miltefosine
Age, years	39 (34–46)	38 (33–47)
Sex, n (%)		
Female	18 (24)	14 (19)
Male	57 (76)	61 (81)
Weight, kg	45 (39–49)	46 (42–49)
Spleen size below costal margin, cm	9 (6–11)	9 (6–13)
Liver size below costal margin, cm	4 (2–6.5)	4 (2–6)
BMI, kg/m^2	17.6 (16.2–18.6)	17.6 (16.7–19.6)
Parasite grading, n (%)		
+6	4 (5)	13 (17)
+5	31 (41)	26 (35)
+4	25 (33)	23 (31)
+3	7 (9)	4 (5)
+2	1 (1)	7 (9)
+1	7 (9)	2 (3)
WBC, × 10^3/µL	2.7 (1.9–3.4)	2.4 (1.8–3.4)
Hemoglobin, g/dL	8.4 (6.9–9.3)	8.8 (7.4–9.9)
Platelets, × 10^3/µL	120 (90–155)	120 (86–173)
Blood urea, mg/dL [10–50]	26 (20–32)	24 (18–30)
Creatinine, mg/dL [0.6–1.1]	0.9 (0.8–1.1)	0.9 (0.8–1.1)
SGOT/AST, U/L [<37]	38 (29–59)	41 (32–56)
SGPT/ALT, U/L [<45]	27 (20–42)	28 (20–38)
Total bilirubin, mg/dL [<1.1]	0.3 (0.3–0.4)	0.3 (0.3–0.5)
VL patient type, n (%)		
Primary	35 (46.7)	46 (61.3)
Relapse	40 (53.3)	29 (38.7)
ART at baseline, n (%)		
No	44 (58.7)	40 (53.3)
Yes	31 (41.3)	35 (46.7)
CD4 count, n (%)		
<50 cells/µL	8 (10.7)	6 (8)
50–100 cells/µL	23 (30.7)	28 (37.3)
100–199 cells/µL	35 (46.7)	29 (38.7)
200–349 cells/µL	7 (9.3)	11 (14.7)
≥350 cells/µL	2 (2.7)	1 (1.3)
Median (IQR), cells/µL	116 (71–162)	106 (72–174)
Viral load, n (%)		
<1000 copies/mL	25 (33.3)	22 (29.3)
1000 to 9999 copies/mL	4 (5.3)	6 (8)
10 000 to 99 999 copies/mL	12 (16)	8 (10.7)
100 000 to 1 000 000 copies/mL	18 (24)	27 (36)
≥1 000 000 copies/mL	16 (21.3)	11 (14.7)
Missing	0	1 (1.3)
Median (IQR), copies/mL	83 364 (210–646 912)	119 842 (128–592 992)
ATT at baseline, n (%)		
No	73 (97.3)	73 (97.3)
Yes	2 (2.7)	2 (2.7)

Both groups' data are medians (IQR) unless indicated otherwise.

Abbreviations: ALT, alanine transferase; ART, antiretroviral therapy; AST, aspartate aminotransferase; ATT, anti-tubercular treatment; BMI, body mass index; IQR, interquartile range; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamate pyruvate transaminas; VL, visceral leishmaniasis; WBC, white blood cell count.

the monotherapy arm (Figure 1). The Kaplan-Meier estimated cumulative incidences of relapse at 6 months and 12 months were fairly similar to estimates of relapse accounting for the competing risk of death and treatment failure (Figures 2 and 3, Supplementary Material 4).

Considering the secondary endpoints, in the monotherapy arm, 93% (n = 70; 95% CI, 90–100%) met the criteria for initial cure at day 29 (including 1 "slow responder"), while 99% (n = 74; 95% CI, 97–100%) did so in the combination arm. At day 390 follow-up, 81% (n = 61; 95% CI, 73–100%) in the

Table 2. Primary Efficacy Endpoint: Intention-to-Treat at Day 210

	AmBisome	AmBisome + Miltefosine
Number of participants	75	75
Number alive and relapse-free at day 210 (%)	64 (85%)	72 (96%)
One-sided 95% confidence interval	(77–100%)	(90–100%)
Treatment failure by day 29, n (%)	4 (5.3%)	1 (1.3%)[a]
Deaths by day 210, n (%)	5 (6.7%)	1 (1.3%)
Relapse by day 210, n (%)	2 (2.7%)	1 (1.3%)

[a]Patient experienced hypersensitivity to AmBisome test dose, was switched to alternative rescue treatment (paromomycin and miltefosine), and was relapse-free at 12 months.

monotherapy arm met the criteria for relapse-free survival; for the combination therapy arm, this was 85% (n = 64; 95% CI, 77–100).

Excluding the 28 participants with TB at time of enrollment, 90% (n = 55/61; 95% CI, 82–100%) and 85% (n = 52/61; 95% CI, 78–100) of those in the monotherapy arm met the relapse-free survival endpoint at day 210 and day 390, respectively; this was 97% (n = 59/61; 95% CI, 91–100%) and 87% (n = 53/61; 95% CI, 80–100%), respectively, in the combination arm. Conversely, for patients considered to have had a diagnosis of TB on enrollment, in the monotherapy arm, 64% (n = 9/14; 95% CI, 43–100%) and 64% (n = 9/14; 95% CI, 43–100%) met the relapse-free survival endpoint at day 210 and day 390, respectively; this was 93% (n = 13/14; 95% CI, 73–100%) and 79% (n = 11/14; 95% CI, 60–100%), respectively, in the combination arm (Figure 4).

Seventy-three (97%) and 74 (99%) patients experienced at least 1 adverse event (AE) in the monotherapy and combination arms, respectively (Table 3, Supplementary Material 5). In the monotherapy arm, 15% (n = 11) of patients experienced at least 1 adverse drug reaction (ADR) possibly related to AmBisome; this was 24% (n = 18) in the combination therapy arm. In addition, 31% (n = 23) of the combination arm had at least 1 ADR possibly related to miltefosine. Adverse events and ADRs were mostly mild, and ADRs that occurred in more than 5% of patients were vomiting (15%, 22 people), which was always possibly related to miltefosine, and hypercreatinemia (9%, 14 people), usually related to AmBisome (Supplementary Material 9). All ADRs were considered expected as per drug reference documentation. 14 severe AEs (SAEs) were reported during the safety monitoring period (9 in monotherapy and 5 in combination therapy), including 4 deaths (all but 1 in the monotherapy arm). No SAE was considered related to the study drugs, while SAEs in all but 1 patient were related to TB (Supplementary Materials 6 and 7).

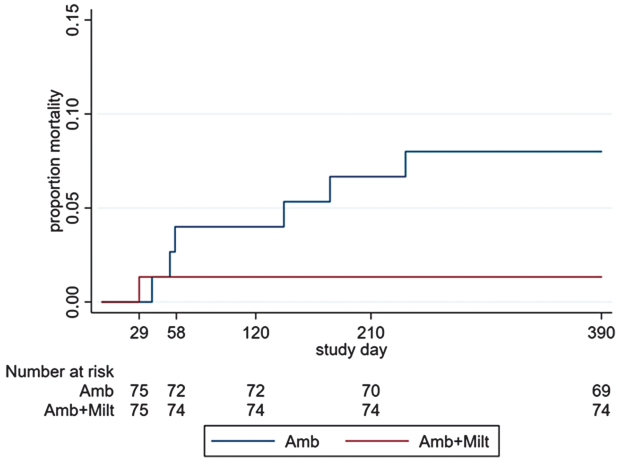


Figure 2. Kaplan-Meier graphs for mortality. Abbreviations: Amb, AmBisome; Milt, miltefosine.

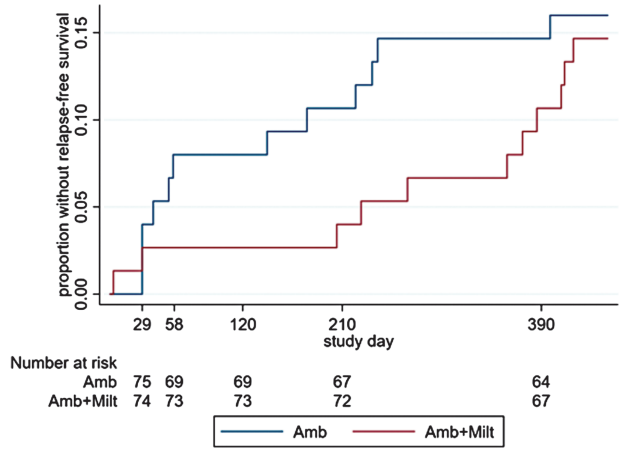


Figure 3. Kaplan-Meier graph for participants without relapse-free survival (ie, treatment failure at day 29, mortality and/or relapse). Abbreviations: Amb, AmBisome; Milt, miltefosine.

Over the course of follow-up, CD4 count recovery followed similar patterns in both arms (Figure 5). The mean increase in CD4 count between initiation of treatment for VL and day 29 was substantial: in the monotherapy arm, the mean increase in CD4 from baseline to day 29 was 98 cells/μL (95% CI, 78–119 cells/μL; paired *t*-test $P < .001$) and in the combination arm was 101 cells/μL (95% CI, 79–122 cells/μL, paired *t*-test $P < .001$). There was no significant difference ($P = .856$) in increase for those already on ART and those initiated on ART within treatment arms after commencement of VL therapy. HIV viral load suppression followed a similar pattern, with the majority of patients meeting the primary and secondary endpoints demonstrating suppression by day 210, which was maintained to day 390. In the combination arm, the risk of relapse among ART failure was 4.8 (95% CI, 1.5–15.8; $P = .03$) times higher than in patients with viral loads of less than 1000 at day 210 or 390. However, within the monotherapy arm, all 4 relapses occurred in patients with undetectable viral load (Supplementary Materials 8 and 9). Patient self-reported ART compliance throughout the study was similarly good in both arms.

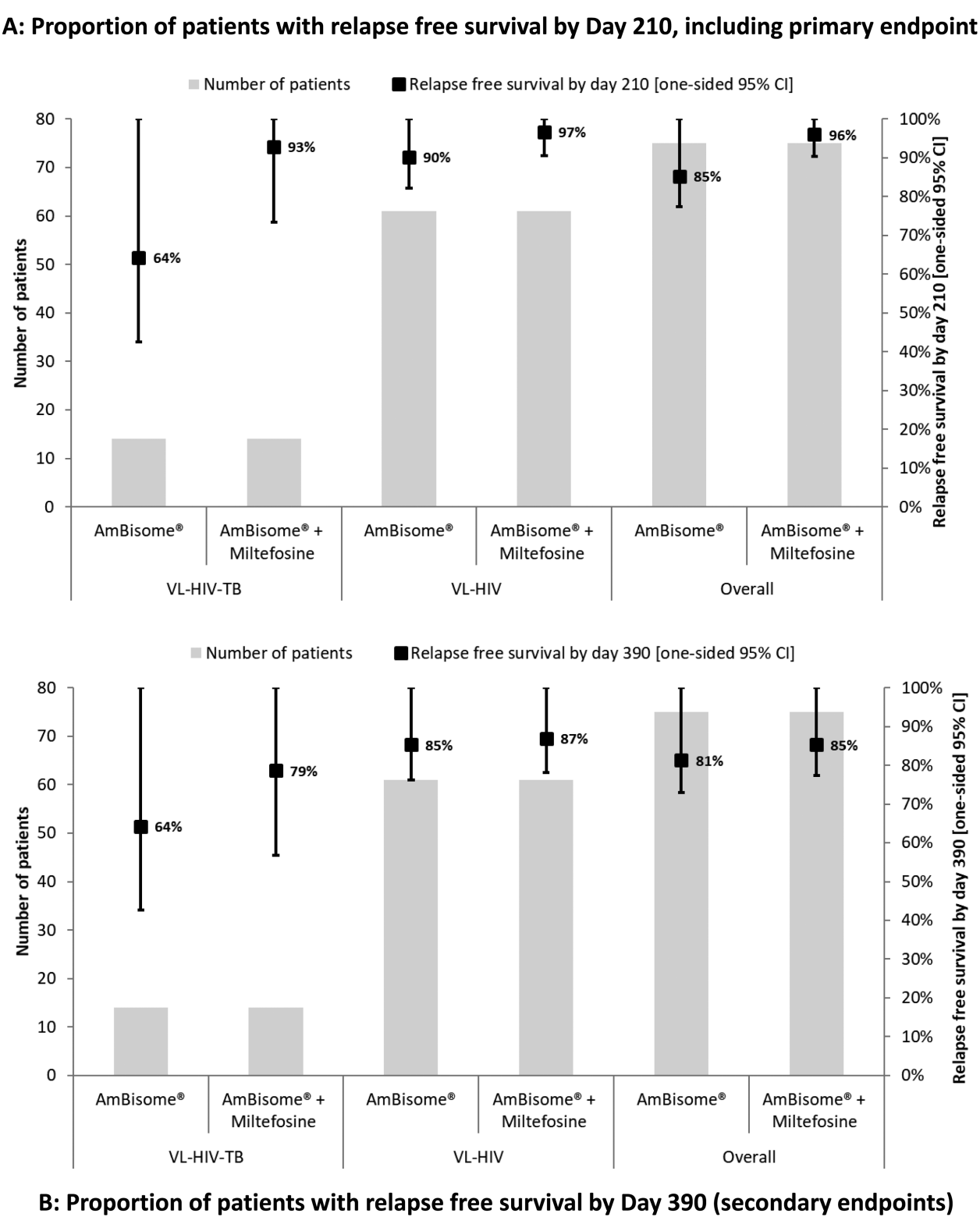


Figure 4. Box plot graph of study endpoints. *A*, Including day 210 primary endpoint. *B*, All day 390 secondary endpoints. Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; TB, tuberculosis; VL, visceral leishmaniasis.

DISCUSSION

Patients with VL-HIV coinfection represent a vulnerable population for whom access to appropriate care is hampered by a pervasive combination of health provider– and community-related stigma, substantial financial barriers, and lack of coordinated multidisciplinary care [16]. This study represents the

Table 3. Adverse Events and Adverse Drug Reactions

	AmBisome (n = 75)	AmBisome + Miltefosine (n = 75)
Patients with at least 1 SAE, n (%)		
Total[a]	6 (8.0)	4 (5.3)
ADR related to AmBisome or miltefosine	0 (0.0)	0 (0.0)
Unrelated to study drug	6 (8.0)	4 (5.3)
Patients with at least 1 AE (whether serious or not), n (%)		
Total[a]	73 (97.3)	74 (98.7)
ADR related to AmBisome	11 (14.7)	18 (24.0)
ADR related to miltefosine	…	23 (30.7)
Unrelated to study drug	73 (97.3)	70 (93.3)
Patients with at least 1 ADR (whether serious or not) by intensity, n (%)		
Mild	9 (12.0)	33 (44.0)
Moderate	2 (2.7)	5 (6.7)
Severe	0 (0.0)	0 (0.0)
Life-threatening	0 (0.0)	0 (0.0)
Number of ADR reports received, n (%) of ADRs		
Total	12 (100)	52 (100)
Mild	10 (83.3)	47 (87.0)
Moderate	2 (16.7)	5 (13.0)
Severe	0 (0.0)	0 (0.0)
Life-threatening	0 (0.0)	0 (0.0)
Number of ADRs per patient		
Median (range)	0 (0–2)	0 (0–4)

Abbreviations: ADR, adverse drug reaction; AE, adverse event; SAE, serious adverse event.

[a]The rows may sum to more than the total because patients may occur in multiple rows.

largest randomized trial of patients with VL-HIV globally to date, and the first of its kind in Asia [8]. This study demonstrated that both treatment regimens appear to be safe and well tolerated, with 85% (n = 64; 95% CI, 77–100%) efficacy at 6 months for AmBisome monotherapy and 96% (n = 72; 95% CI, 90–100%) for the combination of AmBisome and miltefosine.

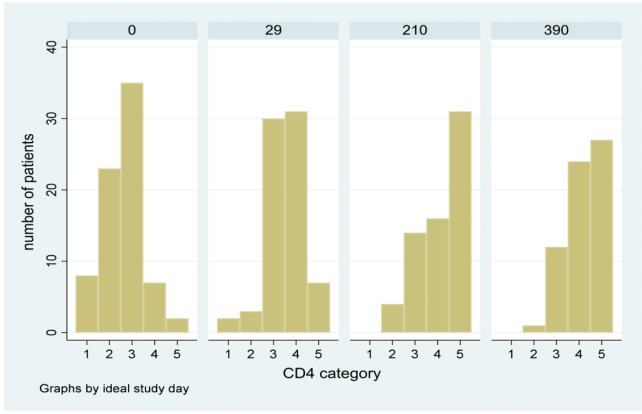
Prior to the current study, the safety and efficacy of the current WHO recommendations of a total dose of 40 mg/kg liposomal amphotericin B had not been evaluated in the Indian subcontinent. This recommendation is based on a randomized trial involving 57 VL-HIV coinfected patients from southern Europe, with *Leishmania infantum*, and compared 2 regimens of amphotericin B lipid complex (15 mg/kg and 30 mg/kg) with meglumine antimoniate. All regimes resulted in initial cure rates below 43% [17]. This current recommendation for intermittent administration over 38 days is challenging for patients in lower- and middle-income countries, for whom lengthy inpatient stays or frequent ambulatory hospital visits result in substantial loss of income for both patient and caregiver [18], and likely increases the risk of nosocomial infection for a severely immunocompromised group in a region with high rates of antimicrobial resistance [19].

Although the high prevalence of TB in advanced HIV is well established, previous studies on VL-HIV treatment have failed to identify and characterize this important subgroup [14]. Outcomes of patients with TB-VL-HIV coinfection appeared worse in both treatment arms; however, they appeared to be particularly poor with AmBisome monotherapy (with the caveat of the small sample size). This is important as the field reality is such that it will be very difficult to effectively diagnose patients with TB in this cohort of patients prior to choosing any particular VL treatment regimen. Additionally, the 19% prevalence of baseline TB infection reported during this study is likely an underestimate of the true prevalence considering that those presenting in critical condition (37%; n = 19/51) were excluded.

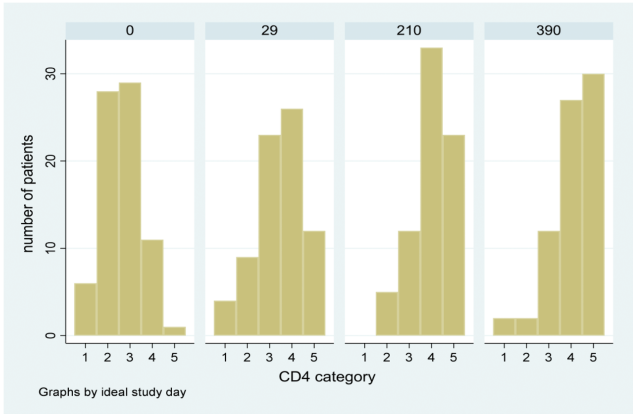
Second, the importance of using viral load to determine the treatment effectiveness of ART (rather than CD4) was robustly demonstrated in this cohort of patients for the first time. Typically, in VL-HIV–endemic areas where access to viral load measurement is limited, low CD4 counts are misinterpreted as poor compliance or ART treatment failure for those established on ART. Instead, by showing that the majority of patients established on ART have viral load suppression at baseline, this study suggests that low CD4 count is related more to coinfection with VL. This is consistent with the rapid increase of CD4 count between VL treatment initiation and day 29.

The main limitation of this study is the noncomparative study design, so that each treatment arm needs to be considered independently of the other, and no direct inferences can be made between the 2 (such as comparing CI crossover): each arm must be considered on its own merit.

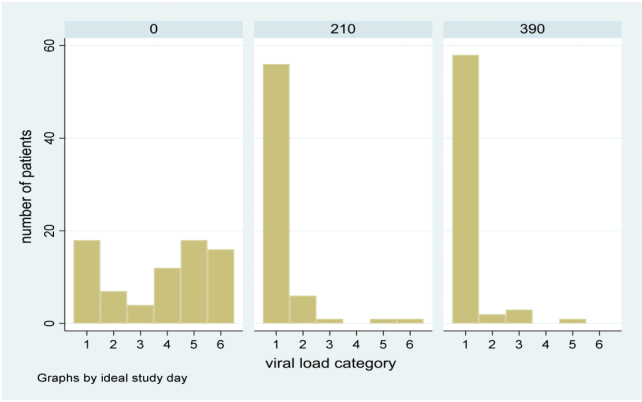
In conclusion, the results of this randomized trial suggest that the use of combination treatment should be considered as



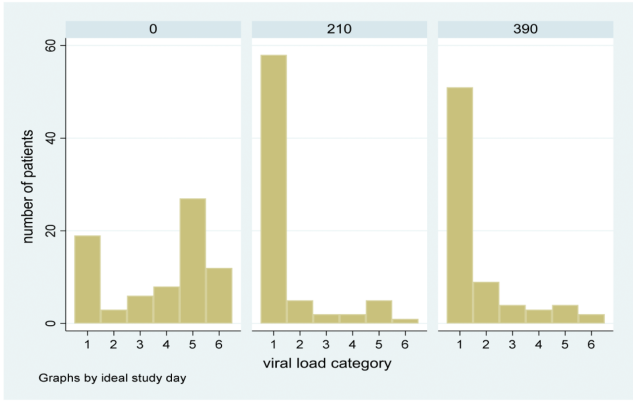
A: CD4-cell counts at baseline, D29, D210 and D390, histogram, Liposomal Amphotericin B monotherapy



B: CD4-cell counts at baseline, D29, D210 and D390, histogram, Liposomal Amphotericin B + Miltefosine



C: Viral load at baseline, D210 and D390, histogram, Liposomal Amphotericin B monotherapy arm



D: Viral load at baseline, D210 and D390, histogram, Liposomal Amphotericin B + Miltefosine arm

Figure 5. *A–D*, Evolution of CD4 count and HIV viral load over 12 months. CD4 categories: Cat 1 = <50, Cat 2 = 50–99, Cat 3 = 100–199, Cat 4 = 200–349, Cat 5 = ≥350 cells/μL. HIV viral load categories: Cat 1 = <150, undetectable; Cat 2 = 150 to <3 $\log_{10}$; Cat 3 = ≥3 to <4 $\log_{10}$; Cat 4 = ≥4 to <5 $\log_{10}$; Cat 5 = ≥5 to <6 $\log_{10}$; Cat 6 = ≥6 $\log_{10}$ copies/mL. Abbreviations: D, day; HIV, human immunodeficiency virus.

a first-line treatment for VL-HIV coinfection in the Indian subcontinent. Further research on cost-effectiveness and patient perceptions of treatment would be valuable.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. S. B. conceived, designed, coordinated the study, and created the first draft of the manuscript. S. K. oversaw site implementation, ethics reporting, and study implementation; R. M. designed and developed all the study tools and supported data analysis and management of the study; N. A. developed the statistical plan, protocol design, and data analysis; D. K. supported the implementation and site management of the study; V. K. supported design of tools and coordinated laboratory components; E. L. supported design and coordination; A. H. and A. d. L. P. supported design, coordination, and implementation; P. D. helped conceive and design the study; N. V., C. S. L., V. N. R. D., and B. R. helped conceive and design the study; V. G., S. R., and F. A. supported data management and independent monitoring of the study; N. G. supported drafting of the manuscript, coordination, and facilitation with the national program; K. P. was Principal Investigator and took overall responsibility for the study. All authors contributed to the final draft of the manuscript.

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