AmBisome monotherapy and combination AmBisome – miltefosine therapy for the treatment of visceral leishmaniasis in patients co-infected with HIV in India: a randomised open label, parallel arm, phase 3 trial

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Summary:

Evidence on treatments for Visceral Leishmaniasis infection in HIV is sparse. This randomised trial showed

that combination AmBisome and miltefosine resulted in 96% (72/75;95%CI 90-100) relapse free survival at day 210; whilst monotherapy with AmBisome resulted in 85% (64/75;95%CI 77-100).

ABSTRACT

Background

Visceral leishmaniasis (VL) in patients living 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 sub-continent.

Methods

This is a randomised open label, parallel arm phase-3 trial conducted within a single hospital in Patna, India. 150 patients aged ≥18 years with serologically confirmed HIV and parasitologically confirmed VL were randomly allocated to one of two treatment arms, either a total 40mg/kg intravenous liposomal amphotericin B(AmBisome) administered in 8 equal doses over 24-days, or a total 30mg/kg intravenous liposomal amphotericin B(AmBisome) administered in 6 equal doses given concomitantly with a total 1.4g oral miltefosine administered through two daily doses of 50mg over 14-days. The primary outcome was ITT relapse-free-survival at day-210, defined as absence of signs and symptoms of VL, or if symptomatic negative parasitological investigations.

Findings

Among 243 patients assessed for eligibility, 150 were recruited between 2ndJanuary 2017 and 5thApril 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;95%CI 90-100) in the combination arm. 19%(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;95%CI 82-100) in the monotherapy and 97%, (59/61;95%CI 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.

Key words: visceral leishmaniasis, liposomal amphotericin B, HIV, miltefosine, India

INTRODUCTION

One-third of all patients living with Human Immunodeficiency Virus (PLHIV) 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 co-infection: like other opportunistic infections in HIV patients, *Leishmania* amastigotes have evolved strategies to survive[4], which are enhanced by HIV co-infection[5] and accelerate progression of disease[6]. This may help explain why the risk of developing VL is estimated to be between 100-2300 times higher in HIV-infected individuals than in those who are HIV-negative[7].

Evidence of treatment for co-infected patients is limited, due to a lack of randomised 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 in almost every respect have consistently been reported in this patient group when compared to patients not known to be HIV-positive.

More recently, evidence from a non-comparative randomised trial of 59 VL-HIV co-infected patients in Ethiopia has been published examining a combination of AmBisome (total dose 30mg/kg in 6 divided doses) with miltefosine (100mg/day for 28 days), and AmBisome monotherapy (total dose 40mg/kg in 8 divided doses over 24 days). This showed poor performance in the monotherapy arm (adjusted efficacy at day 58 of 55% (95%CI 32-78)), whereas the combination arm reached 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 one 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 30 mg/kg in 6 divided doses) given concurrently with oral miltefosine (total dose 1.4g over 14 days in two daily 50mg doses) and intravenous AmBisome monotherapy (total dose 40 mg/kg in 8 divided doses) in Bihar, India.

METHODS

Study design

We designed a parallel arm, open label, randomised, non-comparative phase-3 trial to investigate the safety and efficacy of two treatment regimens for visceral leishmaniasis in patients co-infected 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 timeframe. This also considered that powering for non-inferiority would substantially increase the sample size, while the lack of evidence for the monotherapy arm would prevent meaningful non-inferiority margins to be set. Hence we opted for a non-comparative 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: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 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 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 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. Hypersensitivity to study drugs and those with baseline serum creatinine of >1.2 mg/dl were also excluded.

Randomisation

Subjects were allocated to treatment arm using block randomisation, 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 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 centre for VL. Patients allocated to the liposomal amphotericin B (AmBisome, Gilead Pharmaceuticals, Foster City, California) monotherapy arm were given a total dose of 40 mg/kg administered by slow intravenous (IV) slow infusion of 5mg/kg on days 1-4,8,10,17, and 24 as adapted from current 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 5mg/kg on days 1,3,5,7,9, and 11 concomitantly with a total dose of 1.4g 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, haematological, and biochemical parameters. Where patients were suspected of relapse,

repeat parasitological testing was done. Patients were also monitored for post-kala-azar dermal leishmaniasis (PKDL).

Anti-retroviral 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 six months (day-210) post 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 (i) initial cure at day-29 post start of treatment, defined as cessation of fever, reduction in any initial splenomegaly and a negative parasitological assessment; (ii) relapse-free survival at 12-months (day-390) and (iii) relapse-free survival at day-210 and 390 in the sub-set of patients with no diagnosis of TB co-infection 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 (i.e. up to day-58). Safety assessments were performed at baseline, day 3,10,29 and 58 and grading of event severity was based on the Common Terminology Criteria for Adverse Events (CTCAE), v4.0. CD4 counts were measured at baseline, day-29,210 and 390, while HIV viral load was measured at baseline, day-210 and 390.

Statistical analysis

As the trial was non-comparative, the primary endpoint (relapse-free survival at day-210) was analysed as a proportion for each arm (p). Confidence intervals for this endpoint were calculated using the score (Wilson) method[12]. As the hypothesis was one-sided with significance level 5%, a one-sided 95% confidence interval—p greater than the lower confidence limit—was presented as the primary analysis. A two-sided 90% confidence interval was also calculated. All secondary endpoints were analysed 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 co-infected 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 three infections have different clinical characteristics, and VL-HIV-TB co-infection is relatively unknown[14]. The DSMB recommended increasing 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, i.e. 56, which is 75% of 75, allowing room in case the final proportion of TB cases was slightly higher. A sub-group analysis restricted to those without TB at time of enrolment (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). All adverse events were coded according to the Medical Dictionary for Regulatory Activities (MedDRA, version 23.1).

Results

243 patients were assessed for eligibility between the 2nd January 2017 and 5th April 2018, of whom 42(17%) had negative parasitology and 51(21%) did not meet the inclusion or met exclusion criteria, leaving 150(62%) enrolled (Figure 1) randomised 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 two patients (one patient missing the final AmBisome dose, and one AmBisome hypersensitivity patient 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 anti-retroviral treatment (supplementary material 5); however there were a higher proportion of baseline relapses in the monotherapy arm (53% vs 39%). 19% (n=28) of enrolled participants overall were diagnosed with TB prior to D58; 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 material 2-3), with only 14% (n=21) of all participants having CD4 counts >200 cells/µl. In contrast, 72% (n=47/65) of participants on ART at baseline showed HIV viral load suppression (<1000 copies per ml).

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 1.3%(n=1) in the combination arm (Table 2), with one further death occurring after day 210 in the monotherapy arm (Figure 1). The Kaplan-Meier estimated cumulative incidence 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&3, supplemental material 4).

Considering the secondary endpoints, in the monotherapy arm 93%(n=70) (95%CI 90-100) met the criteria for initial cure at D29 (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 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 enrolment, 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 enrolment, 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).

73 (97%) and 74 (99%) of patients experienced at least one adverse event (AE) in the monotherapy and combination arm respectively (Table 3, supplementary material 5). In the monotherapy arm, 15%(n=11) patients experienced at least one 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 one ADR possibly related to miltefosine. AEs and ADRs were mostly mild, and ADRs occurring in more than 5% of patients were vomiting (15%,22 people), which was always possibly related to miltefosine, and hypercreatinaemia (9%,14 people) usually related to AmBisome (supplemental material 9). All ADRs were considered expected as per drug reference documentation. 14 Severe Adverse Events (SAEs) were reported during the safety monitoring period (9 in monotherapy and 5 in combination therapy), including 4 deaths (all but one in monotherapy arm). No SAE was considered related to the study drugs, while SAEs in all but one patient were related to tuberculosis (supplemental material 6-7).

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, paired *t*-test p<0.001) and in the combination arm 101 cells/µl (95%CI 79-122, paired t-test p<0.001). There was no significant difference (P=0.856) 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 D390. In the combination arm, the risk of relapse among ART treatment failure was 4.8 (95%CI 1.5-15.8; p=0.03) times higher compared to patients with viral loads <1000 at day 210 or 390. However, within the monotherapy arm, all four relapses occurred in patients with undetectable viral load (supplementary materials 8-9). Patient self-reported ART compliance throughout the study was similarly good in both arms.

DISCUSSION

Patients with VL-HIV co-infection 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 largest randomised trial of VL-HIV patients 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) in 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 40mg/kg liposomal amphotericin B had not been evaluated in the Indian subcontinent. This recommendation is based on a randomised trial involving 57 VL-HIV co-infected patients from southern Europe, with *Leishmania infantum*, and compared two regimens of amphotericin B lipid complex (15mg/kg and 30mg/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 characterise this important sub-group[14]. Outcomes of patients with TB-VL-HIV co-infection appeared worse in both treatment arms, however they appeared particularly poor with AmBisome monotherapy (with the caveat of 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 those presenting in critical condition (37%, n=19/51) were excluded.

Secondly, the importance of using viral load to determine 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 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 co-infection 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 non-comparative study design, so that each treatment arm needs to be considered independently of the other, and no direct inferences can be made between the two (such as comparing CI cross over): each arm must be considered on its own merit.

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

NOTES

Contributors:

SB conceived, designed, coordinated the study and created the first draft of the manuscript. SK oversaw site implementation, ethics reporting and study implementation; RM designed, developed all the study tools and supported in data analysis and management of the study; NA developed the statistical plan, protocol design and data analysis; DK supported the implementation and site management of the study; VK supported design of tools, coordinated laboratory components; EL supported design and coordination; AH supported design, coordination and implementation; AdLP supported design, coordination and implementation; PD helped conceive and design the study; NV, CSL and VNRD helped conceive and design the study; BR helped conceive and design the study; VG, SR and FA supported data management and independent monitoring of the study; NG supported in drafting the manuscript, coordination and facilitation with national programme; KP was PI and took overall responsibility for the study. All authors contributed to the final draft of the manuscript.

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Declaration of Interests:

All authors declare no competing interests

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Table 1: Baseline characteristics of both groups

Data are median (inter-quartile range) unless indicated otherwise.

Characteristic (Unit) [normal range]	AmBisome	AmBisome + Miltefosine
Age (years)	39 (34-46)	38 (33-47)
Sex		
Female, n (%)	18 (24)	14 (19)
Male, n (%)	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		
+6, n (%)	4 (5)	13 (17)
+5, n (%)	31 (41)	26 (35)
+4, n (%)	25 (33)	23 (31)
+3, n (%)	7 (9)	4 (5)
+2, n (%)	1 (1)	7 (9)
+1, n (%)	7 (9)	2 (3)
WBC (x10^3/µL)	2.7 (1.9-3.4)	2.4 (1.8-3.4)
Haemoglobin (g/dL)	8.4 (6.9-9.3)	8.8 (7.4-9.9)
Platelets (x10^3/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]	.9 (.8-1.1)	.9 (.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]	.3 (.3-.4)	.3 (.3-.5)
VL patient type		
Primary, n (%)	35 (46.7)	46 (61.3)
Relapse, n (%)	40 (53.3)	29 (38.7)
ART at baseline		
No, n (%)	44 (58.7)	40 (53.3)
Yes, n (%)	31 (41.3)	35 (46.7)
CD4 count (cells/µl)		
<50, n (%)	8 (10.7)	6 (8)
50-100, n (%)	23 (30.7))	28 (37.3)
100 to 199, n (%)	35 (46.7)	29 (38.7)
200 to 349, n (%)	7 (9.3)	11 (14.7)
≥350, n (%)	2 (2.7)	1 (1.3)
Median (IQR)	116 (71-162)	106 (72-174)
Viral Load (copies per ml)		
<1000, n (%)	25 (33.3)	22 (29.3)
1,000 to 9,999, n (%)	4 (5.3)	6 (8)
10,000 to 99,999, n (%)	12 (16)	8 (10.7)
100,000 to 1,000,000, n (%)	18 (24)	27 (36)
≥1,000,000, n (%)	16 (21.3)	11 (14.7)
Missing, n (%)	0	1 (1.3)
Median (IQR)	83,364 (210-646,912)	119,842 (128-592,992)
ATT at baseline		
No, n (%)	73 (97.3)	73 (97.3)
Yes, n (%)	2 (2.7)	2 (2.7)

Table 2: Primary efficacy endpoint: ITT 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%)*
Deaths by day 210: n (%)	5 (6.7%)	1 (1.3%)
Relapse by day 210: n (%)	2 (2.7%)	1 (1.3%)

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

Table 3: Adverse Events and Adverse Drug Reactions

	AmBisome N=75	AmBisome + miltefosine N=75
Number (%) of patients with at least one SAE:		
Total*	6 (8.0)	4 (5.3)
Adverse Drug Reaction (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 one AE (whether serious or not): n (%)		
Total*	73 (97.3)	74 (98.7)
Adverse Drug Reaction (ADR) related to AmBisome	11 (14.7)	18 (24.0)
Adverse Drug Reaction (ADR) related to miltefosine	-	23 (30.7)
Unrelated to study drug	73 (97.3)	70 (93.3)
Patients with at least one 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)
*The rows may sum to more than the total because patients may occur in multiple rows.		

FIGURE LEGENDS

FIGURE 1: TRIAL PROFILE

Figure 2: Kaplan Meier graphs for mortality

Figure 3: Kaplan Meier graph for participants without relapse-free survival (i.e. treatment failure at day 29, mortality and/or relapse)

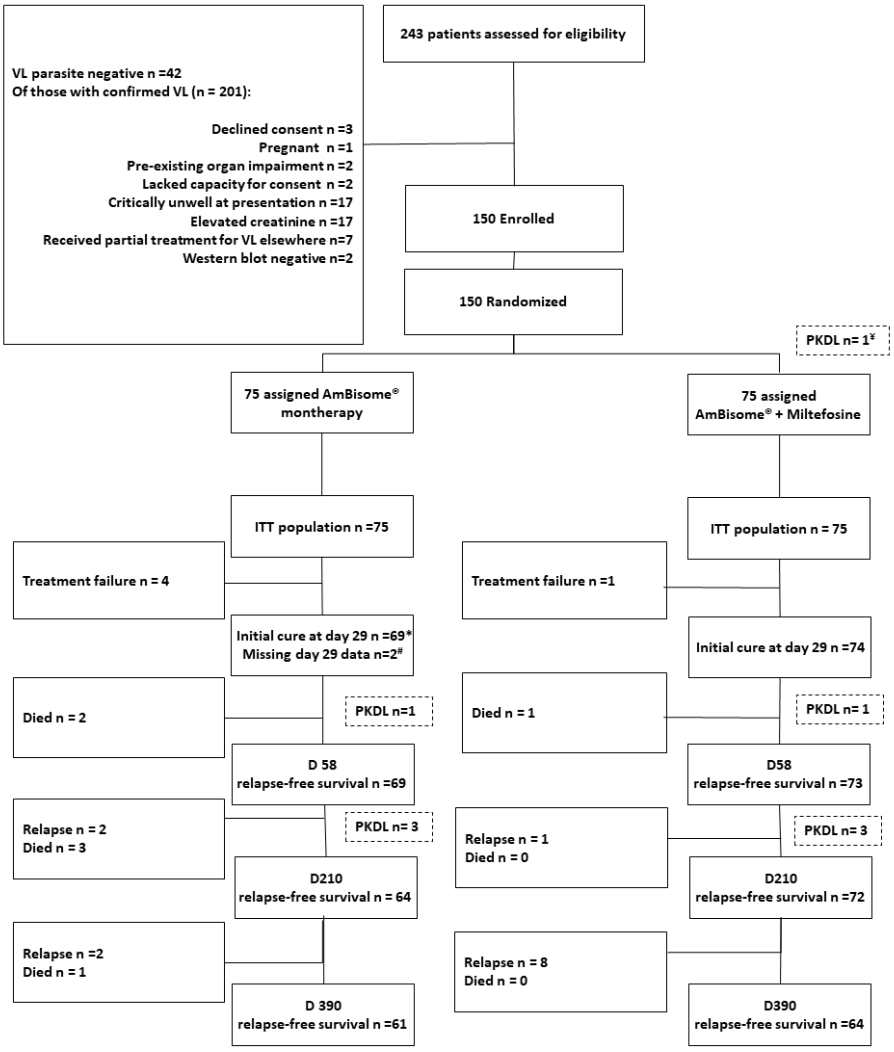
FIGURE 4: Box plot graph of study endpoints; A: Including day 210 Primary endpoint; B: All day 390 secondary endpoints

Figure 5: 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.

Figure 1



¥Simultaneous occurrence of PKDL in active VL at time of recruitment.
#Including one patient who refused final treatment dose of AmBisome and died on day 39.
*Including one 'slow responder'. Patients with clinical improvement but 1+ grading of parasite count at day 29 were considered potential 'slow responders' with aspiration repeated on day 45; if negative are considered D29 initial cure.

Figure 2

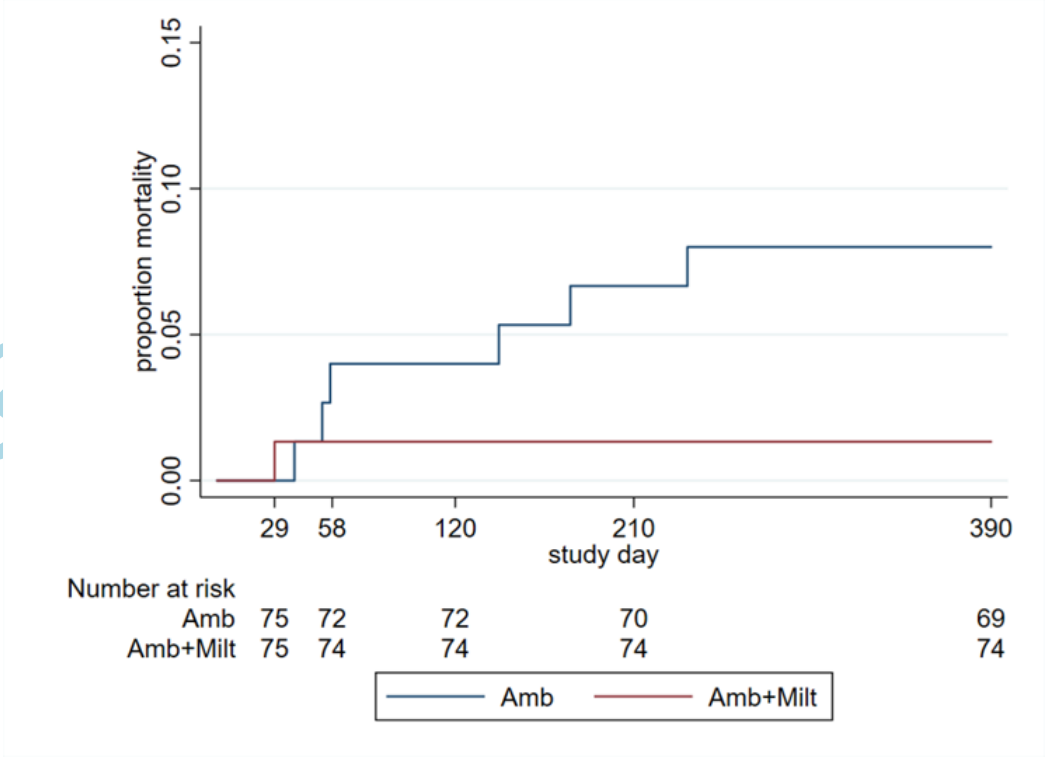


Figure 3

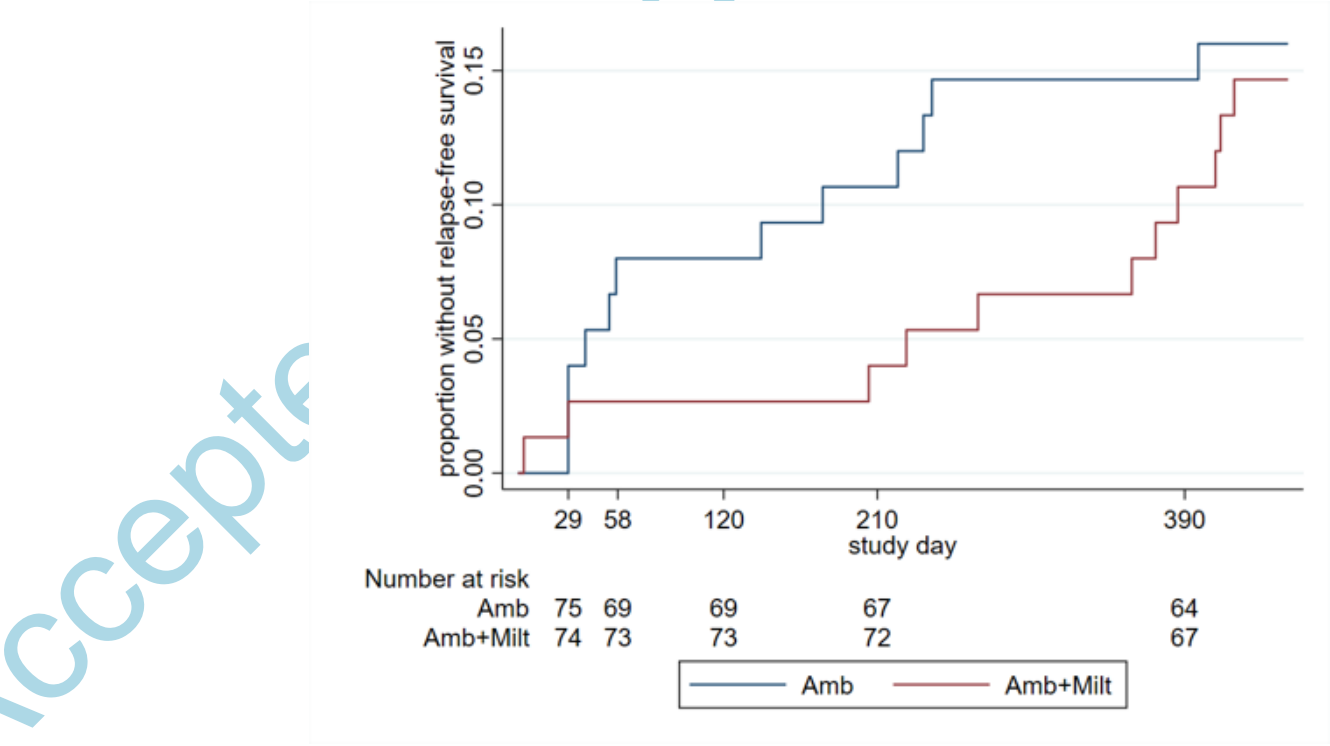


Figure 4

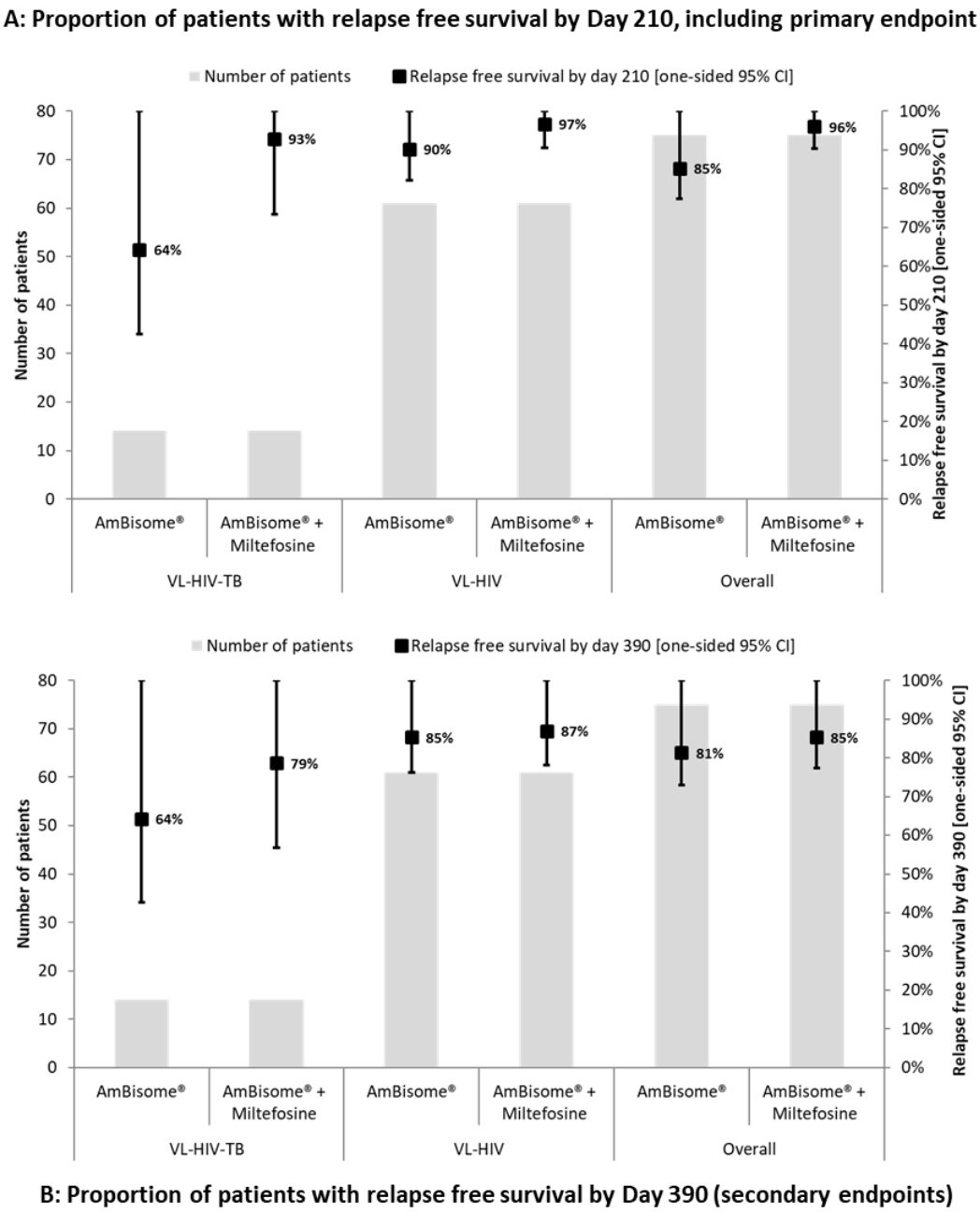
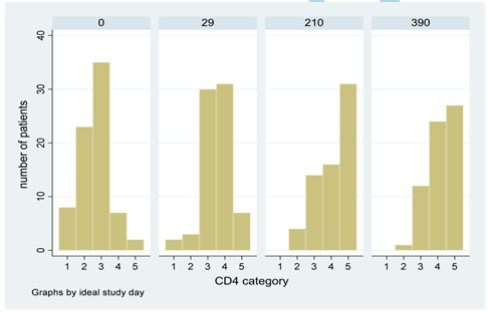
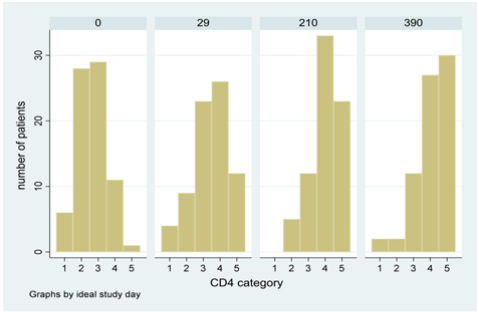


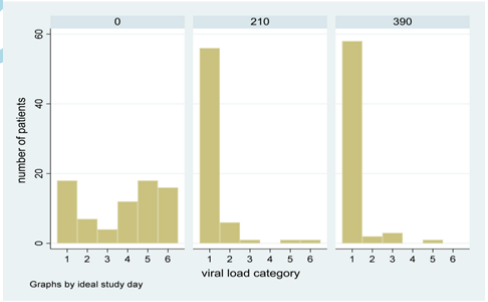
Figure 5



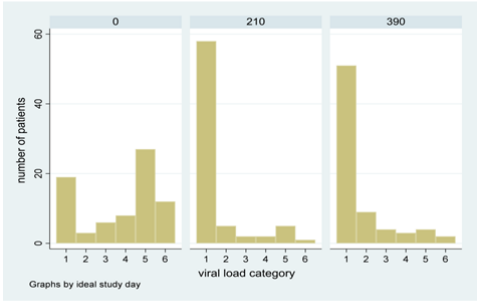
A: CD4-cell counts at baseline, D29, D210 and D390, histogram, Liposomal Amphotericin B monotherapy



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



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