Clinical pharmacology in leishmaniasis: treatment optimization of a neglected disease

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AmBisome® for visceral leishmaniasis patients in East Africa: a multi-centre randomized trial

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Submitted for publication
Abstract

**Background:** Single dose AmBisome® might be a treatment option for visceral leishmaniasis (VL) in Africa but the appropriate dose is unknown.

**Methods:** A multi-centre, open-label, non-inferiority, randomized, controlled trial, with an adaptive design, was conducted to compare the efficacy and safety of a single dose and multiple doses of AmBisome® for the treatment of VL in East Africa. The primary efficacy endpoint was definitive cure (DC) at 6 months. Patients with parasitologically-confirmed, symptomatic, non-severe VL received either a single dose of AmBisome® 7.5 mg/kg body weight, or multiple doses, 7 times 3 mg/kg on days 1-5, 14, and 21. If interim analyses evaluated 30 days after the start of treatment following 40 or 80 patients showed the single dose gave significantly poorer parasite clearance than multiple doses, at the 5% significance level, the single dose was increased by 2.5 mg/kg. In a subset of patients, parasite clearance was also measured using quantitative reverse transcriptase qRT-PCR.

**Results:** The trial was terminated after the third interim analysis due to low efficacy of both regimens. Based on the per-protocol population, DC was 85% (95%CI 73–93%), 40% (95%CI 19–64%), and 58% (95%CI 41–73%) in patients treated with multiple doses (n = 63), and single doses of 7.5 (n = 21) or 10 mg/kg (n = 40), respectively; qRT-PCR suggested superior parasite clearance with multiple doses as early as day 3. Safety data accorded with the drug label.

**Conclusion:** The tested AmBisome® regimens would not be suitable for VL treatment across Africa. An optimal single dose regimen was not identified.

Clinical Trials Registration: www.clinicaltrials.gov NCT00832208
Introduction

Visceral leishmaniasis (VL) is a life-threatening disease and a major health burden in developing countries [1,2]. The World Health Organization (WHO) estimates there are about half a million cases annually, of which 90% occur in South-East Asia, Latin America or East Africa [3]. In East Africa approximately 30,000 people develop symptomatic disease [2] and 4,000 die from VL every year [4].

For decades, the mainstay of VL treatment in Africa has been antimonials, such as sodium stibogluconate (SSG), but this treatment is cardiotoxic [5] and requires a 4-week hospitalisation, imposing a huge economic burden on families [6]. Monotherapy with intramuscular paromomycin (PM) for 3 weeks was shown to be less efficacious in Africa [7] than in Asia [8], but a 17-day treatment with a combination of SSG and PM showed good efficacy and is now recommended as first-line treatment by WHO. However, this treatment also requires a relatively long treatment course and twice daily injections [9]. The currently safest anti-leishmanial drug is AmBisome®, a liposomal amphotericin B formulation with significantly diminished renal toxicity [10]. In trials in India, cure rates of about 90% were obtained with single AmBisome® doses of 5 mg/kg [10]. In addition, 95% efficacy was achieved with higher single doses (10 mg/kg) or when used in combination with miltefosine or Paromomycin [11,12]. Although licensed and recommended for first-line treatment of VL in immunocompetent patients [13], Ambisome use in Africa has been mostly limited to second line treatment in a few centres, due to its high cost and complex administration and storage requirements [14]. A small study with AmBisome® conducted in Kenya indicated higher doses were required than had been used in studies in India. Doses of 2 mg/kg given 3, 5, or 7 times to groups of 10 patients resulted in cure rates of 20%, 90%, and 100%, respectively [15].

The current trial was undertaken to evaluate the efficacy of a simplified dose regimen of AmBisome® for the treatment of symptomatic VL in Africa. The aim of the study was to determine the minimum efficacious and safe single dose, though the likely future use of the drug would be as part of a short course combination treatment regimen designed to reduce costs and improving patient compliance.

Methods

The study design and protocol have been published, in compliance with CONSORT requirements [16]. The protocol was approved in Ethiopia and Sudan by the relevant Institutional / National Ethics Committee and by the Ethics Committee of the London School of Hygiene and Tropical Medicine. The study was conducted in accordance with the declaration of Helsinki, ICH GCP guidelines, and all applicable legal requirements.
Study design

The study was designed as a multi-center, open-label, non-inferiority, randomized, controlled trial, using a sequential-step design to evaluate the efficacy and safety of a single dose treatment regimen of intravenous AmBisome®, compared to the reference multiple dose regimen currently approved in the US: 3 mg/kg body weight on days 1 to 5, 14, and 21. The single dose tested in the first cohort was 7.5 mg/kg body weight. Patients were randomized to receive either treatment using a computer-generated randomisation list, stratified by site. Individual treatment allocations were placed in sealed, opaque envelopes which were opened after a patient had been entered into the trial. Two interim analyses were planned, after enrolment of 20 and 40 patients per arm, to detect ineffectual single doses early. If the stopping rule was met, the single dose was increased by 2.5 mg/kg and recruitment into the two arms restarted. The multiple dose treatment remained the same throughout (Figure 1). Non-responders to treatment were considered treatment failures and received rescue medication (full multiple dose AmBisome® regimen for single-dose failures and SSG for multiple dose failures).

Study population

Patients of at least 4 years of age, confirmed HIV-negative, with parasitologically-confirmed non-severe VL, were enrolled in three centers: (1) Gondar University
Hospital, Amhara Regional State, Northern Ethiopia; (2) Arba Minch Hospital, Gama Gofa, Southern Nations, Nationalities and Peoples Regional State, Southern Ethiopia; and per protocol amendment (3) Ministry of Health Hospital, Kassab, Gedaref State, Eastern Sudan from May 2009 to September 2010.

Endpoints

Primary and secondary efficacy endpoints were definitive and initial cure rates based on the absence of parasites in tissue (bone marrow, lymph node or spleen) aspirates after 6 months and on day 30, respectively. Secondary safety endpoint was the incidence rate of treatment emergent adverse events (TEAE), which were classified according to the Medical Dictionary for Regulatory Activities (MedDRA). Safety was further evaluated based on laboratory analyses of blood samples collected at baseline and on days 2 to 5, 7, 14, 21 and 30, and at 3 and 6 months. In Kassab, per protocol amendment, peripheral blood samples were also analysed for parasite loads in a subset of 5 consenting patients in each of the 10mg/kg single dose and multiple dose arms. For this, a validated quantitative reverse-transcriptase polymerase-chain-reaction (qRT-PCR) method targeting Leishmania 18S ribosomal RNA was used [17]. Genetic material was extracted using a modified Boom-method [17–19]. qRT-PCR analysis using a Bio-Rad CFX-96 real-time machine (Bio-Rad, Veenendaal, the Netherlands) was performed at Koninklijk Instituut voor de Tropen (KIT).

Sample Size

For the primary endpoint non-inferiority comparison, 120 patients per arm would provide 80% power to detect non-inferiority within a margin of 10%, assuming 95% cure in the reference arm, a one-sided alpha of 0.05 and 15% loss-to-follow-up. In interim analyses, 20 patients per arm would provide 90% power to detect a difference of at least 35% in parasite clearance rates at day 30, assuming 95% cure in the reference arm and a two-sided alpha of 0.05. With 40 patients per arm, there would be 90% power to detect a difference of at least 25% under the same assumptions.

Statistical Analysis

Interim analyses were based on day 30 cure in the Intention-to-Treat (ITT) population. Decision-making at each interim analysis was based on a test of difference between the parasite clearance rates in the single dose arm and multiple dose arms. If the single dose arm showed significantly poorer efficacy (p<0.05), the single-dose was increased prior to re-starting recruitment. Patients allocated to the multiple dose arm for discontinued single-dose comparisons were not included in comparisons of higher single doses.
Table 1. Baseline data on patient demographics, clinical characteristics and laboratory values.

<table>
<thead>
<tr>
<th></th>
<th>Multiple dose 21 mg/kg n = 63</th>
<th>Single dose 7.5 mg/kg n = 21</th>
<th>Single dose 10 mg/kg n = 40</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age in years</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Children (4 – 17y), n (%)</em></td>
<td>37 (59)</td>
<td>4 (19)</td>
<td>25 (63)</td>
</tr>
<tr>
<td><em>Adults (≥ 18y), n (%)</em></td>
<td>26 (41)</td>
<td>17 (81)</td>
<td>15 (37)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>10 (16)</td>
<td>2 (11)</td>
<td>10 (25)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>53 (84)</td>
<td>19 (91)</td>
<td>30 (75)</td>
</tr>
<tr>
<td><strong>Spleen size in cm</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>9.5 (5.3)</td>
<td>12.0 (6.0)</td>
<td>9.1 (5.3)</td>
</tr>
<tr>
<td><strong>Nutritional status†</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight [kg], mean (SD)</td>
<td>36 (14.5)</td>
<td>44 (12.2)</td>
<td>33 (14.4)</td>
</tr>
<tr>
<td>Severe underweight, n (%)</td>
<td>18 (29)</td>
<td>9 (43)</td>
<td>11 (28)</td>
</tr>
<tr>
<td>Underweight, n (%)</td>
<td>22 (35)</td>
<td>7 (33)</td>
<td>17 (43)</td>
</tr>
<tr>
<td>Normal weight, n (%)</td>
<td>22 (35)</td>
<td>5 (24)</td>
<td>12 (30)</td>
</tr>
<tr>
<td><strong>Hemoglobin (g/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>7.9 (1.7)</td>
<td>7.7 (1.6)</td>
<td>7.7 (1.4)</td>
</tr>
<tr>
<td><strong>AST</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>52 (27.1)</td>
<td>48 (28.5)</td>
<td>54 (29.0)</td>
</tr>
<tr>
<td><strong>ALT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>32 (20.7)</td>
<td>35 (21.3)</td>
<td>33 (20.0)</td>
</tr>
<tr>
<td><strong>Parasite count (log scale)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6+, n (%)</td>
<td>2 (3)</td>
<td>2 (10)</td>
<td>0</td>
</tr>
<tr>
<td>5+, n (%)</td>
<td>7 (11)</td>
<td>5 (23)</td>
<td>7 (18)</td>
</tr>
<tr>
<td>4+, n (%)</td>
<td>14 (22)</td>
<td>6 (29)</td>
<td>4 (10)</td>
</tr>
<tr>
<td>3+, n (%)</td>
<td>15 (23)</td>
<td>4 (19)</td>
<td>9 (22)</td>
</tr>
<tr>
<td>2+, n (%)</td>
<td>11 (17)</td>
<td>4 (19)</td>
<td>9 (22)</td>
</tr>
<tr>
<td>1+, n (%)</td>
<td>12 (20)</td>
<td>0</td>
<td>10 (25)</td>
</tr>
<tr>
<td>0 or missing, n (%)</td>
<td>2* (3)</td>
<td>0</td>
<td>1** (3)</td>
</tr>
</tbody>
</table>

AST, aspartate aminotransferase; ALT, alanine aminotransferase
† classified using weight for height and BMI for age in those aged ≤19 years and BMI in those aged >19: normal if -2SDs weight for height or BMI for age ≤+1SD or 18.5 ≤ BMI <25.0; underweight if -3SDs weight for height or BMI for age ≤-2SD or 16.0 ≤ BMI <18.5; severely underweight if weight for height or BMI for age ≤-3SD or BMI<16.0
* 1 case of unconfirmed VL with no parasites detected (major protocol deviation, excluded from analysis) and one case of no parasite count recorded on a log scale in which VL was confirmed by lymph node aspirate.
** no parasite count recorded on a log scale in which VL was confirmed by lymph node aspirate.
Key assumptions for the planned final analysis were not met due to low efficacy in the multi-dose arm and the trial was terminated prematurely (see Results). Cumulative data for each treatment regimen were used to calculate the percentage of patients cured, with exact binomial 95% confidence intervals (CI), at day 30 and 6 months follow-up in ITT and per-protocol (PP) analysis populations. Complete-case analysis was used to account for missing outcome data (patients with missing data excluded). For safety, the number and percentage of patients per arm experiencing AEs were presented, for AEs with cumulative incidence higher than 10%.

For the parasite clearance from peripheral blood, a linear mixed effects regression model using the natural log-transformed parasite loads was applied to estimate the time to clear 50% and 90% of parasites for each individual. Model performance and significance were assessed by analysis of variance (ANOVA).

Results

Early termination

The first interim analysis took place as planned, comparing the 7.5 mg/kg single dose to the multiple dose regimen with 20 and 18 patients per arm, respectively, in the two Ethiopian sites. As the stopping rule was met (Table 2: Fisher’s exact test, p=0.015), the single dose was increased to 10 mg/kg, and recruitment restarted at both Ethiopian sites and in an additional site, Kassab, Sudan. There was not a significant difference in efficacy found at the next interim analysis, comparing 10 mg/kg to the multiple dose arm (p=0.748), but when 44 patients had been recruited into the multiple dose and 40 patients into the 10 mg/kg single-dose arm, the third interim analysis suggested unexpectedly low initial cure rates in both arms; 84% in the multiple dose versus 73% in the single-dose arm. The stopping rule was not met (chi-squared test, p=0.196), but based on the observed poor efficacy overall, and following discussions with the data safety and monitoring board (DSMB) and investigators, the sponsor terminated the trial.

At that time, a total of 124 patients had been enrolled, 63 had received the multiple dose regimen, 20 a single dose of 7.5 mg/kg dose and 41 a single dose of 10 mg/kg.

Baseline characteristics

The most common presenting VL symptoms were fever and weight loss, followed by loss of appetite, abdominal swelling, and cough; less commonly observed were epistaxis, diarrhea, and skin lesions. Other characteristics of patients at entry to the trial are summarized in Table 1. Overall 82% of patients were male and about
Table 2. Interim analyses and non-comparative efficacy analysis for primary (day 210) and secondary (day 30) endpoints

<table>
<thead>
<tr>
<th></th>
<th>Multiple dose 7x 3 mg/kg</th>
<th>Single dose 7.5 mg/kg</th>
<th>Single dose 10 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of patients</td>
<td>No. cured (%)</td>
<td>95% CI</td>
</tr>
<tr>
<td><strong>Parasite clearance at Day 30</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interim analysis 1 c</td>
<td>18</td>
<td>16 (89)</td>
<td>65 - 99</td>
</tr>
<tr>
<td>Interim analysis 2 d</td>
<td>25</td>
<td>19 (74)</td>
<td>55 - 91</td>
</tr>
<tr>
<td>Interim analysis 3 e</td>
<td>44</td>
<td>37 (84)</td>
<td>70 - 93</td>
</tr>
<tr>
<td><strong>Cure at Day 30</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>62 f</td>
<td>53 (85)</td>
<td>74 - 93</td>
</tr>
<tr>
<td>Kassab, Sudan</td>
<td>18</td>
<td>16 (89)</td>
<td>65 - 99</td>
</tr>
<tr>
<td>Gondar, Ethiopia</td>
<td>20</td>
<td>13 (65)</td>
<td>41 - 85</td>
</tr>
<tr>
<td>Arba Minch, Ethiopia</td>
<td>24</td>
<td>24 (100)</td>
<td>86 - 100 b</td>
</tr>
<tr>
<td><strong>Cure at Day 210 (6 months follow-up)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>54 f</td>
<td>46 (85)</td>
<td>73-93</td>
</tr>
<tr>
<td>Kassab, Sudan</td>
<td>17</td>
<td>13 (76)</td>
<td>50-93</td>
</tr>
<tr>
<td>Gondar, Ethiopia</td>
<td>14</td>
<td>10 (71)</td>
<td>42-92</td>
</tr>
<tr>
<td>Arba Minch, Ethiopia</td>
<td>23</td>
<td>23 (100)</td>
<td>83-100 b</td>
</tr>
</tbody>
</table>

Intention-to-Treat and Per-Protocol complete-case analysis populations identical at day 30 & day 210.

a Exact binomial 95% confidence interval (CI)

b One-sided 97.5% confidence interval

c Multiple versus 7.5mg/kg single dose; p = 0.015 (Fisher’s exact test): Dose escalation rule met; increase dosage to 10mg/kg & continue recruitment

d Multiple versus 10mg/kg single dose; p = 0.748 from chi-square test of difference between arms: Dose escalation rule not met; continue recruitment (same dosage in single-dose arm)

e Includes patients in interim analysis 2. Multiple versus 10mg/kg single dose; p-value = 0.196 from chi-square test of difference between arms: Dose escalation rule not met; concerns arose regarding low cure in each arm and recruitment not continued

f 8 patients lost to follow-up by day 210 (all from the multiple-dose arm)
half were children. About two thirds of patients were underweight or severely underweight. Mean hemoglobin concentrations were <8.0 g/dl, and anemia was common, but neither baseline laboratory parameters nor vital signs showed any major difference among dose groups. Baseline characteristics were generally comparable in the multiple and the 10 mg/kg single dose group, whereas the smaller 7.5 mg/kg dose group showed some imbalances: patients in this group were older and accordingly had higher body weight and larger spleen size, but also the highest baseline parasitemia. Patients were younger in Kassab (10.5±5.0 years) than in Gondar (20.9±6.3) or Arba Minch (17.4±10.2) and more often female in Kassab

<table>
<thead>
<tr>
<th>Table 3. Infusion-related and drug-related treatment emergent AEs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total number of patients randomised</strong></td>
</tr>
<tr>
<td><strong>n = 63</strong></td>
</tr>
<tr>
<td><strong>Number of patients with any infusion-related AE, n (%)</strong></td>
</tr>
<tr>
<td><strong>Infusion related AE by MedDRA preferred term:</strong></td>
</tr>
<tr>
<td>Vomiting</td>
</tr>
<tr>
<td>Chills</td>
</tr>
<tr>
<td>Pyrexia</td>
</tr>
<tr>
<td>Arthralgia</td>
</tr>
<tr>
<td>Back Pain</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td><strong>Number of patients with TEADR, n (%)</strong></td>
</tr>
<tr>
<td><strong>(with cumulative incidence of 10% or greater in any group)</strong></td>
</tr>
<tr>
<td>TEADR by MedDRA preferred term:</td>
</tr>
<tr>
<td>Anaemia</td>
</tr>
<tr>
<td>Thrombocytosis</td>
</tr>
<tr>
<td>Pyrexia</td>
</tr>
<tr>
<td>Alanine aminotransferase increased</td>
</tr>
<tr>
<td>Asparate aminotransferatase increased</td>
</tr>
<tr>
<td>Blood creatine increased</td>
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<tr>
<td>Blood magnesium abnormal</td>
</tr>
<tr>
<td>Blood magnesium decreased</td>
</tr>
<tr>
<td>Blood potassium decreased</td>
</tr>
<tr>
<td>Blood sodium decreased</td>
</tr>
<tr>
<td>Hypermagnesaemia</td>
</tr>
<tr>
<td>Hypokalaemia</td>
</tr>
<tr>
<td>Hypomagnesaemia</td>
</tr>
<tr>
<td>Arthralgia</td>
</tr>
<tr>
<td>Azotaemia</td>
</tr>
<tr>
<td>Renal impairment</td>
</tr>
</tbody>
</table>

AE, adverse event; TEADR, treatment emergent adverse drug reaction; treatment emergent = onset between day 1 and day 60 inclusive; adverse drug reaction if investigator judged relationship to treatment as "probable", "possible" or "unlikely".

* This includes patients with infusion related reactions.

b Renal impairment: 3 mild cases and 1 moderate as graded by investigator, all resolved during study period
(33.3%) than in Gondar (10.5%) or Arba Minch (12%). In Arba Minch, they were more often normal weight (42.0%) than in Gondar (21.1%) or Kassab (28.6%).

**Efficacy**

In the first section of Table 2, parasite clearance rates at day 30 are shown for the three interim analyses. Below this, summary data for the parasite clearance rate at day 30 and the cure rate at 6 months are shown for all patients and for those treated at each of the 3 centers. The IC and DC rates with the standard multiple dose treatment were both 85%. IC rates with single doses of 7.5 and 10 mg/kg were 50% and 73%, respectively, and DC rates were lower, at 40% and 58%, respectively. However, there were variations in treatment response between treatment centers, with poor efficacy in Kassab and Gondar, particularly with single doses. By contrast, at Arba Minch the multiple doses, as well as the single dose of 10 mg/kg, resulted in complete cure and treatment failures were observed with the 7.5 mg/kg dose only. All non-responders were cured after receiving rescue medication.

**Safety**

TEAE were common, regardless of dose regimen. Severity was mostly mild or moderate and only about 2% of TEAE were rated severe, mostly with respect to laboratory measurements. There was only one non-fatal SAE, a pneumonia deemed unlikely related to the drug, and one unrelated death due to snakebite. AEs for which relatedness could not be excluded are listed in Table 3. These potential adverse drug reactions were seen in the three dose groups and occurred in both the multiple and the higher single dose group with similar frequencies.

**Pharmacodynamics**

At baseline, semi-quantitative microscopy counts on bone marrow aspirates correlated well with parasite loads in peripheral blood assessed by qRT-PCR ($R^2$: 0.77, p<0.01). Mean natural log-normalized parasite loads (P) were comparable in the single and multiple dose groups (6.4 lnP/mL, 95%CI: 4.6 - 8.2 versus 5.1 lnP/mL, 95%CI: 3.3 – 6.8; p = 0.358) at baseline. Three out of the 5 patients of each group had baseline blood parasite loads >50 per mL and thus had clearance rates assessed and modelled over the first 7 days. Mean clearance rate constants were significantly different between the single and the multiple dose group (0.35 per day, 95%CI: 0.00 – 0.70 versus 1.14 per day, 95%CI: 0.78 – 1.50; p = 0.012) as early as day 3 (Figure 2), corresponding to mean parasite elimination half-lives of 1.97 days (95% CI: 0.99 – 278) for the single-dose and 0.61 days (95% CI: 0.46 – 0.89) for the multiple dose group. Times required for 90% parasite clearance were estimated at 6.55 (95% CI: 3.29 – 923) and 2.02 (95% CI: 1.53 – 2.95) days, respectively. One patient of the single dose group had a low blood parasite load at baseline, which increased until day 30,
Discussion

We investigated the efficacy of AmBisome® given as multiple or single dose regimens for treatment of VL in Africa. The aim was to determine the minimum efficacious dose and treatment safety in HIV-negative patients. However, the study had to be prematurely terminated due to unacceptable efficacy of both the single and the multiple dose treatments, with a cure rate of only 85% in the multiple-dose arm. Adverse effects of treatment in this study were in line with the current drug label.

The low efficacy was unexpected, as total doses of 10mg/kg and above resulted in DC rates of at least 90% in a trial in Kenya [15]. We have no explanation for the poor responses seen in this study or for the observed geographical variations in response. Methodological bias is unlikely, as similar variation in treatment response in these three sites was seen for daily doses of 11 mg/ kg body weight paromomycin base over 21 days [7], a regimen which had also proven efficacious in India [8]. The poor response may relate to characteristics of either the host or the parasite or a combination of both.

Differences in baseline patient characteristics among the three sites may have contributed to variations in treatment response. In Arba Minch, only about 16% of patients were severely underweight, as compared to more than twice as many in

![Figure 2. Parasite clearance from peripheral blood. Multiple Ambisome® doses (black) versus single Ambisome® dose (grey). n = 3 per arm; data are given as geometric means (+ half of the 95% confidence interval)](image-url)
the two sites with poorer responses. However, in all sites, between 58% and 79% of patients were underweight or severely underweight. As poor treatment response was not restricted to the severely underweight only, mechanisms are likely to be more complex. Furthermore, the single dose trial in India did not have stricter inclusion/exclusion criteria (it included patients with severe malnutrition) and had a comparable population in terms of mean age and weight [11]. It is possible that other factors, such as hemoglobin and immunity, may play a role; however, it is unlikely that they can account for the large differences seen between India and East Africa or within Africa.

Regarding the parasite, susceptibility to treatments (e.g. SSG) is known to be different in Africa and India, although development of resistance is unlikely to account for the poor response in this study. In Africa, resistance has generally not been an issue yet and amphotericin B has not been widely used. Out of 21 pathogenic species of Leishmania, only one species complex is supposed to actually cause VL, of which two close species are known, L. donovani and L. infantum and only the former exists in Africa. Recent studies employing microsatellite markers have revealed the existence of genetically varied populations of L. donovani in southern and northern Ethiopia [20]. However, whether such genetic variations account for the differences in treatment outcomes remains to be determined. Currently ongoing drug susceptibility testing may give some clues. Due to early termination of the study, results were not conclusive regarding the primary objective, i.e. the efficacy comparison of single and multiple doses. However, pharmacodynamic data suggests a benefit of multiple doses on peripheral parasite clearance. On day 3 of treatment, differences in peripheral parasite clearance were significant, even though total administered doses up to this time point were comparable in both arms (10 versus 9 mg/kg, respectively). This suggests a possible role for the frequency of administration. This would need to be confirmed by further studies, but is supported by the relatively high cure rate of failing single dose patients rescued by the additional standard multiple dose regimen. Even in India, where efficacy of single doses was shown to be good, it was lower than with multiple dose regimens [21,22]. In Africa, there might be no universal dosing regimen for VL and those proven efficacious elsewhere might first need to be personalized, based on relevant factors of general health and early predictors of response. Also in this respect, qRT-PCR results are interesting, although its use in Africa might be hampered by relatively low blood parasitemia at baseline and prohibitive cost. More sensitive prognostic pharmacodynamic biomarkers, applicable in all patients, are needed to monitor treatment response.
Conclusion

Efficacy of AmBisome® for the treatment of VL in East Africa was variable and overall low as compared to India. Current recommendations from a WHO expert committee report are to use only high multiple dose regimens accumulating to a total dose of 30 mg/kg as second line treatment [23]. The best first line treatment in Africa remains the combination of SSG and PM.

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