Short Course High-dose Liposomal Amphotericin B for HIV-associated Cryptococcal Meningitis: A phase-II Randomized Controlled Trial

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Summary: This phase-II randomized trial showed that single high-dose (10mg/kg) Liposomal Amphotericin B (L-AmB) treatment was well tolerated and non-inferior in terms of fungal clearance to standard 14-day L-AmB (3mg/kg) treatment in patients with HIV-associated cryptococcal meningitis.

Abstract

Background: Cryptococcal meningitis (CM) causes 10-20% of HIV-related deaths in Africa. We performed a phase-II non-inferiority trial examining the Early Fungicidal Activity (EFA) of three short-course, high-dose liposomal amphotericin B (L-AmB) regimens for CM in Tanzania and Botswana.

Method: HIV-infected adults with CM were randomized to: (i) L-AmB 10mg/kg day 1 (single dose); (ii) L-AmB 10mg/kg day 1, 5mg/kg day 3 (two doses); (iii) L-AmB 10mg/kg day 1, 5 mg/kg days 3 and 7 (three doses); (iv) standard 14-day L-AmB 3mg/kg/day (control); all given with fluconazole 1200mg/day for 14 days. Primary endpoint was mean rate of clearance of cerebrospinal fluid (CSF) cryptococal infection (EFA). Non-inferiority was defined as an upper limit of the two-sided 95% confidence interval (CI) of difference in EFA between intervention and control less than 0.2 $\log_{10}$CFU/ml/day.

Results: 80 participants were enrolled. EFA for daily L-AmB was -0.41 (standard deviation 0.11, n=17) $\log_{10}$CFU/mL/day. Difference in mean EFA from control was -0.11 (95%CI -0.29,0.07) $\log_{10}$CFU/mL/day faster with single dose (n=16); -0.05 (95%CI -0.20,0.10) $\log_{10}$CFU/mL/day faster with two doses (n=18); and -0.13 (95%CI -0.35,0.09) $\log_{10}$CFU/mL/day faster with three doses (n=18). EFA in all short-course arms was non-inferior to control at the predefined non-inferiority margin. Overall 10-week mortality was 29% (n=23) with no statistical difference between arms. All arms were well tolerated.

Conclusions: Single dose 10mg/kg L-AmB was well tolerated and led to non-inferior EFA compared to 14 days of 3mg/kg/d L-AmB in HIV-associated CM. Induction based on single 10mg/kg L-AmB dose is being taken forward to a phase-III clinical endpoint trial.

Keywords: Cryptococcal meningitis, HIV, Ambisome, amphotericin, randomized clinical trial

Introduction

Early mortality in HIV treatment programmes in low-resource settings is considerably higher than in high-income countries[1-4]. Up to 20% of these deaths are directly attributable to cryptococcal meningitis (CM)[2, 5, 6], which was estimated to cause 181,100 deaths globally in 2014[6]. The poor outcomes reported using currently available antifungal therapy are a critical driver of this high CM-related mortality. Mortality using amphotericin B deoxycholate-based therapy in low-resource settings, even in clinical trials, remains in the region of 35-45% at 10 weeks[7-10]. Recommended amphotericin B deoxycholate-based therapy requires hospitalization for at least 14 days, and its toxicity profile requires costly laboratory monitoring[11]. In most resource limited settings, the lack of access to reliable laboratory monitoring, limited nursing capacity, and inadequate funding, means that amphotericin B deoxycholate is not routinely available. As a consequence oral fluconazole monotherapy is widely used but even at a high dose of up to 1200mg/day it is much less rapidly fungicidal than amphotericin B and mortality at 10 weeks is around 60%[12, 13]. New treatment strategies are urgently needed.

Liposomal amphotericin B (L-AmB) has lower rates of drug induced toxicities than AmB deoxycholate[14]. Although L-AmB is recommended as treatment for HIV-associated CM in several national guidelines[15, 16], optimal regimens are unknown. The long tissue half-life and effective penetration into the brain tissue suggest it may be possible to deliver effective treatment with very short courses of high-dose L-AmB[17, 18]. Pharmacokinetic data from animal models and humans suggest that increasing L-AmB dosing from the currently recommended 3-4mg/kg may lead to improved outcomes, and that very short course regimens may be as effective as daily therapy[17, 19]. The concept of single or intermittent dose L-AmB therapy has been tested in prophylaxis for haematology patients, with single doses of up to 15mg/kg given without significant toxicities[20-22], and is established in treatment of visceral leishmaniasis where single doses of 10mg/kg are routinely given and have been shown to be efficacious[23].

The strategy of short-course, high dosing of L-AmB for HIV-associated CM has not been previously tested in a clinical trial. We performed an open label phase II randomized non-inferiority trial to

compare alternative short course L-AmB regimens for the treatment of HIV-associated CM. Our aim was to determine which, if any, of the three alternative schedules of intermittent high-dose L-AmB could be adopted for the development of a phase III randomized controlled clinical endpoint trial. We measured the effects on early fungicidal activity (EFA), which is associated closely with all-cause mortality[9, 24, 25].

Materials and Methods

The trial protocol has previously been published in full[26]. The study was carried out at Princess Marina Hospital, Gaborone, Botswana and Bugando Medical Centre and Sekou Toure Hospital, Mwanza, Tanzania. The study was approved by the Research Ethics Committees of the London School of Hygiene and Tropical Medicine, the University of Pennsylvania, the Botswana Ministry of Health (HRDC) and the National Institute of Medical Research (NIMR) Tanzania. The study was conducted in accordance with the principles of International Conference of Harmonisation (ICH) good clinical practice (GCP) and was prospectively registered on the International Standard Randomized Controlled Trial Register (ISRCTN 10248064).

Participants and procedures

Between October 2014 and September 2016 sequential HIV-infected adults $\geq$ 18 years with a first episode of CM, diagnosed by cerebrospinal fluid (CSF) India ink or cryptococcal antigen (CrAg) lateral flow assay (IMMY, Norman, Oklahoma, USA), were screened for enrolment in the trial. Pregnant or lactating patients, patients with a previous serious reaction to study drugs, or patients on antifungal treatment for more then 48 hours were excluded. Patients who were both ART naïve and ART exposed were recruited. Written informed consent was obtained from participants, or in the case of mental obtundation, from a guardian or person with legal responsibility. Patients with mental obtundation were re-consented on recovery.

Patients were block randomized individually to one of four treatment groups by means of random computer generated lists with an allocation ratio of 1:1:1:1 and block sizes of 8. Randomizations lists were created by an independent statistician who prepared sealed envelopes in advance that were sent to the sites. Trial pharmacists were responsible for radomization at each site. Randomization was stratified by abnormal mental status (Glasgow Coma Scale (GCS) of 15 or <15) and ART status on admission at each site. The patients and clinical trial team were not blinded. Laboratory staff performing quantitative fungal cultures were blinded to treatment allocation.

The four treatment arms were 1) L-AmB (AmBisome, Gilead Sciences Inc.) 10mg/kg day one (single dose); 2) L-AmB 10mg/kg day one and 5mg/kg day three (two doses); 3) L-AmB 10mg/kg day one, 5mg/kg days three and seven (three doses); and 4) L-AmB 3mg/kg/day for 14 days (control). L-AmB was given by intravenous infusion over two hours. All patients also received 1200mg/day oral fluconazole (Diflucan, Pfizer or Medopharm Fluconazole) for the first two weeks. Unless contraindicated all patients received one litre of 0.9% normal saline with 20 mmol of KCl prior to L-AmB to minimise nephrotoxicity and were routinely given oral potassium (16 mmol KCl twice daily) and magnesium (11 mmol Mg^{2+} once daily) supplementation and daily trimethoprim-sulfamethoxazole prophylaxis. After the two week induction phase patients received fluconazole 800mg/day until 10 weeks and 200mg/day thereafter. ART consisting of tenofovir, emtricitabine, and efavirenz was commenced four to six weeks after initiation of antifungal therapy in individuals not already on ART.

Evaluations and outcomes

At baseline patients underwent a lumbar puncture (LP) for opening pressure, cell count and differential, protein, glucose, India Ink, CrAg, quantitative fungal culture and routine bacterial culture. LPs for opening pressure measurements and CSF samples for quantitatative fungal culture, were repeated on treatment days 3, 7 and 14. Patients with a CSF opening pressure greater than 30cm H_2O

or symptoms of raised intracranial pressure underwent daily LPs to remove CSF in accordance with guidelines[15]. Quantitative cryptococcal cultures were plated in serial ten-fold dilution and the dilution with the least colonies, but at least 30 colony forming units (CFUs) per 200μL, was used to calculate CFU/mL quantitative cryptococcal cultures results, as previously described[24]. A linear regression of $\log_{10}$ CFU/mL against time was calculated for each patient. All data points were analysed except sterile cultures in the second week if these values lessened the slope, as sterility would have been achieved before that day's LP and using the second week value would therefore underestimate the true slope[9, 24].

All participants had baseline blood tests including full blood count, urea, creatinine, electrolytes, alanine transaminase (ALT), HIV test (if status unknown), and CD4 count. During the two week induction phase patients underwent alternate day renal function and electrolyte assessment and twice weekly monitoring of FBC and ALT. Clinical and laboratory adverse events were graded using the NIH DAIDS Toxicity Table[27]. Clinical response was monitored daily for the first two weeks or until discharge (whichever was later) then in a follow-up clinic 3, 4, 6, and 10 weeks after starting therapy.

The primary outcome measure was the mean rate of decrease in CSF cryptococcal CFU, also known as Early Fungicidal Activity (EFA) of each L-AmB treatment arm. Secondary outcome measures were mortality at two and ten weeks; proportion of patients in each treatment arm suffering clinical and laboratory-defined grade III/IV adverse events; and median percentage change from baseline in laboratory defined parameters.

Statistical analysis

Using a non-inferiority design, assuming an EFA of 0.50 $\log_{10}$ CFU/mL/day with a standard deviation of 0.25 $\log_{10}$ CFU/mL/day in the standard daily dosing arm, with a pre-specified acceptable delta of 0.2 $\log_{10}$ CFU/mL/day, one-sided alpha of 0.025 and 90% power, gave a sample size of 33 patients per arm. The pre-specified delta of 0.2 $\log_{10}$ CFU/mL/day was selected on the basis of prior evidence showing increased mortality once EFA falls below 0.3 $\log_{10}$ CFU/mL/day (i.e. the projected 0.50 $\log_{10}$ CFU/mL/day in the control arm minus pre-specified delta of 0.2 $\log_{10}$ CFU/mL/day)[26]. A sample size of 40 patients per arm (160 patients in total) was planned to allow for patients who died prior to obtaining EFA measurement. An interim analysis was planned after 80 participants were randomized in the study. The primary analysis was based on the intention-to-treat (ITT) population. Patients who died before having a repeat LP on day three or those with a negative baseline culture could not have an EFA calculated and were therefore not included the EFA analysis, but were analysed for secondary endpoints. Linear regression models were used with the mean rate of decrease in $\log_{10}$ CSF cryptococcal CFU (EFA) being the dependent variable and the treatment groups (using the control group as a comparator) the primary independent variables. The short-course L-AmB groups were compared to the control arm for non-inferiority using the pre-specified delta of 0.2 $\log_{10}$ CFU/mL/day. Statistical significance was defined as $p \leq 0.05$. Following an unadjusted EFA analysis, adjusted analysis was performed including covariates that may determine outcomes (baseline fungal burden, CD4 cell count, abnormal mental status, sex, age, and ART status) giving summary differences with 95% confidence intervals (CIs). Grade III and IV adverse events were tabulated by study arm, and the overall number of adverse events compared using the chi-squared test. The proportion of patients experiencing grade III and IV anaemia, renal impairment, and hypokalaemia during 2-week induction treatment was compared across study arms using the chi-squared test. Mean change in haemoglobin and percentage change in creatinine during 2-week induction therapy were compared across study arms using analysis of variance (ANOVA) analysis and chi-squared testing respectively. Mortality was compared across groups using chi-squared testing. Data were analysed using Stata, version 13 (StataCorp, College Station, TX).

Role of the funding source

The study was funded through a Gilead Investigator Initiated Award (IN-EU-131-D036). The funding source and drug manufacturers had no involvement in the study design, in the collection, analysis and interpretation of data, in the preparation of manuscripts, or the decison to submit this paper for publication. The authors had full access to all study data and had final responsibility for the decision to submit for publication.

Results

The study was stopped on the recommendation of the independent Data Monitoring Committee (DMC) at the pre-planned interim analysis as the primary objective had been achieved, with non-inferiority achieved in all three study arms at both the pre-defined 95% confidence level and the stringent 99% confidence level, and no safety concerns with short-course treatment, with the recommendation that the trial proceed onto the clinical endpoint phase III trial. At the time of stopping, 134 patients had been screened for the trial. Fifty four patients were excluded (Figure 1), and 80 patients enrolled and randomized to one of the four treatment groups: 18 to single dose, 20 to two doses, 21 to three doses, and 21 to control. One patient was excluded after randomization after it emerged they had been treated for a previous episode of CM; thus 79 patients completed the study, with no loss to follow-up during the initial two-week induction phase. All participants received the treatment as per the randomization arm. One patient was lost to follow-up between two and ten weeks. Baseline clinical and laboratory characteristics were well balanced between treatment groups (Table 1). Thirty two percent of patients (25) were on ART at presentation with CM, the median baseline CD4 count was 32 cells/μL, and 28% (22) had a GCS <15.

Primary Outcome

EFA was calculated for 69 patients (17 in the control group, 16 in the single dose group, 18 in the two dose group, and 18 in the three dose group). Five patients died prior to follow-up LP and 5 patients had negative baseline cultures precluding EFA calculation. All the short course, high-dose arms of L-AmB were non-inferior in terms of EFA to 14 days of standard dose L-AmB at the pre-defined non-inferiority margin of 0.2 $\log_{10}$CFU/mL/day (Figure 2a). The mean (SD) EFA was -0.41 (0.11) $\log_{10}$CFU/mL/day with standard treatment (control), -0.52 (0.35) $\log_{10}$CFU/mL/day with single dose L-AmB, -0.47 (0.29) $\log_{10}$CFU/mL/day with two doses, and -0.54 (0.44) $\log_{10}$CFU/mL/day with three doses. The difference in mean EFA between single dose and control was -0.11 (95% CI -0.29 to 0.07) $\log_{10}$CFU/mL/day; between two doses and control was -0.05 (95% CI -0.20 to 0.10) $\log_{10}$CFU/mL/day; and between three doses and control was -0.13 (95% CI -0.35 to 0.09) $\log_{10}$CFU/mL/day. There was no evidence for any dose response effect with additional L-AmB doses, suggesting maximal fungicidal activity was achieved with a single 10mg/kg dose. This remained the case when the analysis was adjusted for factors that have previously been shown to affect EFA (CSF fungal burden and CD4 count), abnormal mental status, and also sex, age and ART status (Figure 2c).

Mortality

Overall all-cause mortality rates were 15% (12/79) at two weeks and 29% (23/79) at ten weeks, with no significant difference between treatment arms. Two-week mortality was 10% (2/21) in the control arm, 11% (2/18) in the single dose arm, 15% (3/20) in the two dose arm, and 25% (5/20) in the three dose arm (p = 0.52). At ten weeks mortality was 29% (6/21) in the control arm, 22% (4/18) in the single dose arm, 15% (3/20) in the two dose arm, and 50% (10/20) in the three dose arm (p = 0.09) (Table 2). Mortality at ten weeks was associated with abnormal mental status at baseline in univariable analysis (OR 3.75, 95% CI 1.3-10.7), but not with baseline fungal burden, baseline CD4 count, or ART status. The mortality difference between the single dose and control arms was 6.4% (95% CI -21% to 34%).

Safety

There were no safety concerns with short-course treatment in terms of fungal clearance and no patients receiving short course L-AmB required additional "rescue" L-AmB therapy. The three high-dose short-course L-AmB regimens were all well tolerated. Eighty eight grade III and above adverse events (AEs) occurred in 47 patients: 45 grade III and 43 grade IV/V AEs, with no significant differences observed between treatment arms (Table 3). Of these, 49 were clinical, and 39 laboratory AEs. There were ten grade III and two grade IV AEs which were attributed to treatment with L-AmB, all of which were expected L-AmB related side effects (3 grade III hypokalemia, 1 grade IV hypokalemia, 1 grade III hypomagnasemia, 4 grade III creatinine rises, 1 grade III and 1 grade IV anaemia, 1 grade IV hyponatraemia). Both grade IV L-AmB related events occured in the control group. During induction therapy grade III and IV anaemia occurred in 6% (5) and 1% (1) overall, renal impairment in 5% (4) and 1% (1) overall, and hypokalaemia in 1% (1) and 1% (1) overall, with no significant differences between treatment arms (Table 2).

Eleven trial participants were readmitted to hospital during the 10-week follow-up period, at a median of 41 days (IQR 25-55 days), including 4 in the control arm, 4 in the single dose arm, none in the two dose arm, and three in the three dose arm. Cryptococcal immune reconstitution inflammatory syndrome (IRIS) was suspected or diagnosed in 5 patients (11%) of the 45 patients initiating ART, 2 of whom died, with no significant differences between study arms.

Discussion

The use of a single 10mg/kg dose of L-AmB was non-inferior to standard 3mg/kg daily dosing for 14 days in reducing CSF cryptococcal burden in patients with a first episode of HIV-associated cryptococcal meningitis. These findings are consistent with previous human and animal studies demonstrating that shorter courses of amphotericin based treatment may be better tolerated and as effective as conventional 14 day courses[13, 17, 28-30].

High dosages of liposomal amphotericin B were well tolerated, and the safety profile of all liposomal amphotericin B regimens tested compared favourably to data from prior clinical trials using conventional amphotericin B deoxycholate in similar patient populations, both in terms of mortality at 10 weeks and drug induced toxicities[9, 11]. Overall rates of adverse events associated to L-AmB were very low, with just one patient (1%) developing grade IV anaemia during induction therapy (in the control arm), compared to 18% of a historic cohort of 368 CM patients receiving amphotericin B deoxycholate treatment and an identical pre-hydration and electrolyte supplementation regimen to that used in the current trial[11]. The median fall in haemoglobin during the first two weeks of treatment was 0.9 g/dL, compared to 2.3 g/dL in the previous cohort of amphotericin B deoxycholate treated patients[11], and there was a median increase in creatinine of 14% over the initial two weeks, compared to 73% in the amphotericin B deoxycholate treated cohort[11]. There were no grade IV adverse events attributed to high dose L-AmB during the trial. Rates of recurrence of CM symptoms and IRIS were low, with suspected IRIS events occurring in 11% of individuals initiated on ART during the trial.

Based on these phase-II results, single dose 10mg/kg L-AmB is being taken forward to a phase-III clinical endpoint trial (ISRCTN 72509687). Given the correlation between EFA and clinical outcome[9, 25], the rapid EFA seen with single 10mg/kg doses of L-AmB should result in a clinically efficacious alternative treatment for CM. The ten-week mortality rate of 22% with the single dose 10mg/kg L-AmB selected for study in the phase III trial, and the overall mortality rate of 29% in the trial, compare favourably with mortality rates of approximately 40% seen in recent large clinical trials of 2-week amphotericin B deoxycholate based treatment[7-9]. Notably, these mortality rates were in the context of fluconazole as a second antifungal agent. The addition to high dose L-AmB of a more efficacious agent such as flucytosine, which has been proven to be superior to fluconazole in the recent ACTA trial[30], may enable a further reduction in mortality rates.

The current phase-II study was not powered to detect a mortality difference, as shown by the wide 95% confidence intervals around the mortality difference, and as expected no significant difference in mortality between the four L-AmB treatment arms was seen. The higher mortality rate in the three-dose arm were likely due to chance alone, with 40% (4) of the deaths occurring prior to receipt of the third dose of L-AmB.

In conclusion, we have demonstrated that a single 10mg/kg dose of liposomal amphotericin B given in combination with high dose fluconazole is non-inferior to daily dosed liposomal amphotericin B at the standard dose of 3mg/kg plus high dose fluconazole in terms of rate of fungal clearance in patients with HIV-associated cryptococcal meningitis. This short-course treatment strategy is now being tested against amphotericin B deoxycholate in a clinical endpoint trial. If confirmed to be effective, single high dosages of liposomal amphotericin B given with an optimised oral antifungal medication backbone would provide a feasible, well tolerated, and sustainable treatment regimen for HIV-associated CM in resource limited settings where the safe administration of amphotericin B deoxycholate treatment is not possible. Reductions in the need for toxicity monitoring, fewer drug related adverse events, and the potential for shorter periods of hospitalisation are likely to mean that a single high dose L-AmB treatment strategy is cost effective, and a highly favourable alternative to the current standard of care.

Author's contributions

JNJ and TSH conceptualized and designed the study, supervised implementation, analysed the data, and drafted the final manuscript. TBL, AAC, GB, MM, RKKP, and MWT implemented the study. KT and NL were the research nurses, CM implemented the laboratory aspects of the trial, and NM was the study pharmacist. JK and JC supervised implementation. DL drafted the initial manuscript. WH assisted with conceptualized and designed of the study and critically reviewed the manuscript. SM was the trial manager, assisted with study design, supervised implementation and data management, and helped draft the final manuscript. All authors reviewed and approved the final manuscript.

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Declaration of Interests

JNJ and TH were recipients of a Gilead Investigator award. TH declares consultancy fees from Viamet, lecture fees from Pfizer and Gilead sciences, and money from Immuno-Mycologics.. WH holds or has recently held research grants with F2G, AiCuris, Astellas Pharma, Spero Therapeutics, Matinas Biosciences, Antabio, Amplyx, Allecra, Auspherix and Pfizer. He holds awards from the National Institutes of Health, Medical Research Council, National Institute of Health Research, and the European Commission (FP7 and IMI). WH has also received personal fees in his capacity as a consultant for F2G, Amplyx, Ausperix, Spero Therapeutics, Medicines Company, Gilead and Basilea. WH is Medical Guideline Director for the European Society of Clinical Microbiology and Infectious Diseases, and an Ordinary Council Member for the British Society of Antimicrobial Chemotherapy. GB declares consultancy fees from Pfizer, grants and travel expenses from NIH, and lecture payments from ViralEd. Other authors: None to declare.

References

1. Braitstein P, Brinkhof MW, Dabis F, et al. Mortality of HIV-1-infected patients in the first year of antiretroviral therapy: comparison between low-income and high-income countries. Lancet **2006**; 367(9513): 817-24.

2. Lawn S, Harries A, Anglaret X, Myer L, Wood R. Early Mortality among adults accessing antiretroviral treatment programmes in sub-Saharan Africa. AIDS (London, England) **2008**; 22(15): 1897-908.

3. Amuron B, Namara G, Birungi J, et al. Mortality and loss-to-follow-up during the pre-treatment period in an antiretroviral therapy programme under normal health service conditions in Uganda. BMC Public Health **2009**; 9: 290.

4. Gupta A, Nadkarni G, Yang WT, et al. Early mortality in adults initiating antiretroviral therapy (ART) in low- and middle-income countries (LMIC): a systematic review and meta-analysis. PLoS ONE **2011**; 6(12): e28691.

5. Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS (London, England) **2009**; 23(4): 525-30.

6. Rajasingham R, Smith RM, Park BJ, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. The Lancet infectious diseases **2017**; pii: S1473-3099(17)30243-8.

7. Day JN, Chau TT, Wolbers M, et al. Combination antifungal therapy for cryptococcal meningitis. The New England journal of medicine **2013**; 368(14): 1291-302.

8. Beardsley J, Wolbers M, Kibengo FM, et al. Adjunctive Dexamethasone in HIV-Associated Cryptococcal Meningitis. The New England journal of medicine **2016**; 374: 542-54.

9. Jarvis JN, Bicanic T, Loyse A, et al. Determinants of mortality in a combined cohort of 501 patients with HIV-associated Cryptococcal meningitis: implications for improving outcomes. Clin Infect Dis **2014**; 58(5): 736-45.

10. Boulware DR, Meya DB, Muzoora C, et al. Timing of antiretroviral therapy after diagnosis of cryptococcal meningitis. The New England journal of medicine **2014**; 370(26): 2487-98.

11. Bicanic T, Bottomley C, Loyse A, et al. Toxicity of Amphotericin B Deoxycholate-Based Induction Therapy in Patients with HIV-Associated Cryptococcal Meningitis. Antimicrobial agents and chemotherapy **2015**; 59(12): 7224-31.

12. Longley N, Muzoora C, Taseera K, et al. Dose response effect of high-dose fluconazole for HIV-associated cryptococcal meningitis in southwestern Uganda. Clin Infect Dis **2008**; 47(12): 1556-61.

13. Jackson A, Nussbaum J, Phulusa J, et al. A Phase II Randomised Controlled Trial Adding Oral Flucytosine to High Dose Fluconazole, with Short-course Amphotericin B, for Cryptococcal Meningitis in Malawi. AIDS (London, England) **2012**; 26(11): 1363-70.

14. Hamill RJ, Sobel JD, El-Sadr W, et al. Comparison of 2 doses of liposomal amphotericin B and conventional amphotericin B deoxycholate for treatment of AIDS-associated acute cryptococcal meningitis: a randomized, double-blind clinical trial of efficacy and safety. Clin Infect Dis **2010**; 51(2): 225-32.

15. Perfect JR, Dismukes WE, Dromer F, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the infectious diseases society of america. Clin Infect Dis **2010**; 50(3): 291-322.

16. Nelson M, Dockrell D, Edwards S, et al. British HIV Association and British Infection Association guidelines for the treatment of opportunistic infection in HIV-seropositive individuals 2011. HIV medicine **2011**; 12 Suppl 2: 1-140.

17. Lestner J, McEntee L, Johnson A, et al. Experimental Models of Short Courses of Liposomal Amphotericin B for Induction Therapy for Cryptococcal Meningitis. Antimicrobial agents and chemotherapy **2017**.

18. Vogelsinger H, Weiler S, Djanani A, et al. Amphotericin B tissue distribution in autopsy material after treatment with liposomal amphotericin B and amphotericin B colloidal dispersion. The Journal of antimicrobial chemotherapy **2006**; 57(6): 1153-60.

19. O'Connor L, Livermore J, Sharp AD, et al. Pharmacodynamics of liposomal amphotericin B and flucytosine for cryptococcal meningoencephalitis: safe and effective regimens for immunocompromised patients. The Journal of infectious diseases **2013**; 208(2): 351-61.

20. Gubbins PO, Amsden JR, McConnell SA, Anaissie EJ. Pharmacokinetics and buccal mucosal concentrations of a 15 milligram per kilogram of body weight total dose of liposomal amphotericin B administered as a single dose (15 mg/kg), weekly dose (7.5 mg/kg), or daily dose (1 mg/kg) in peripheral stem cell transplant patients. Antimicrobial agents and chemotherapy **2009**; 53(9): 3664-74.

21. Mehta P, Vinks A, Filipovich A, et al. High-dose weekly AmBisome antifungal prophylaxis in pediatric patients undergoing hematopoietic stem cell transplantation: a pharmacokinetic study. Biol Blood Marrow Transplant **2006**; 12(2): 235-40.

22. Cornely OA, Maertens J, Bresnik M, et al. Liposomal amphotericin B as initial therapy for invasive mold infection: a randomized trial comparing a high-loading dose regimen with standard dosing (AmBiLoad trial). Clin Infect Dis **2007**; 44(10): 1289-97.

23. Sundar S, Chakravarty J, Agarwal D, Rai M, Murray HW. Single-dose liposomal amphotericin B for visceral leishmaniasis in India. The New England journal of medicine **2010**; 362(6): 504-12.

24. Brouwer AE, Rajanuwong A, Chierakul W, et al. Combination antifungal therapies for HIV-associated cryptococcal meningitis: a randomised trial. Lancet **2004**; 363(9423): 1764-7.

25. Bicanic T, Muzoora C, Brouwer AE, et al. Independent association between rate of clearance of infection and clinical outcome of HIV-associated cryptococcal meningitis: analysis of a combined cohort of 262 patients. Clin Infect Dis **2009**; 49(5): 702-9.

26. Molefi M, Chofle AA, Molloy SF, et al. AMBITION-cm: intermittent high dose AmBisome on a high dose fluconazole backbone for cryptococcal meningitis induction therapy in sub-Saharan Africa: study protocol for a randomized controlled trial. Trials **2015**; 16: 276.

27. "U.S. Department of Health and Human Services NIoH, National Institute of Allergy and Infectious Diseases, Division of AIDS". Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.1. . **2017**.

28. Muzoora CK, Kabanda T, Ortu G, et al. Short course amphotericin B with high dose fluconazole for HIV-associated cryptococcal meningitis. The Journal of infection **2011**; 64(1): 76-81.

29. Livermore J, Howard SJ, Sharp AD, et al. Efficacy of an abbreviated induction regimen of amphotericin B deoxycholate for cryptococcal meningoencephalitis: 3 days of therapy is equivalent to 14 days. MBio **2013**; 5(1): e00725-13.

30. Molloy S, Kanyama C, Heyderman R, et al. Antifungal Combinations for Treatment of Cryptococcal Meningitis in Africa. N Engl J Med. 2018 Mar 15;378(11):1004-1017.

Figure Legends

Figure 1: Consort Diagram

Figure 2: Early Fungicidal Activity (EFA) by treatment group (log_{10}CFU/mL/day). (A): Difference in mean EFA between intervention arms and control. All 3 short-course treatment arms were non-inferior to control. (B) Individual patient slopes over the initial 14 days of treatment. The mean slope (standard deviation) is given below each plot. Sterile cultures in the second week that lessened the

slope and were excluded from EFA calculation as sterility would have been achieved before that day's LP are shown in the dotted grey line. (C) Adjusted difference in mean EFA between intervention arms and control. All 3 short-course treatment arms remained non-inferior to control when adjusted for i) baseline fungal burden (QCC); ii) baseline CD4 count; iii) baseline mental status; iv) QCC and CD4 count; v) QCC, CD4 count and mental status and vi) QCC, CD4 count, mental status, sex, age, and ART status.

Table 1: Baseline Characteristics of Trial Participants

	All (n=79)	**Control (n=21)**	**Single Dose L-AmB (n=18)**	**Two Doses L-AmB (n=20)**	**Three Doses L-AmB (n=20)**
Age, years Median (IQR)	**38 (32-43)**	39 (34-46)	37 (32-40)	37 (30-43)	38 (33-43)
Sex % male (n)	**54% (43)**	57% (12)	67% (12)	50% (10)	45% (9)
Weight, kg Median (IQR)	**52 (45-61)**	52 (48-65)	52 (43-65)	52 (45-55)	56 (45-68)
On ART % on ART at presentation (n)	**32% (25)**	38% (8)	22% (4)	35% (7)	30% (6)
Currently on TB Treatment % (n)	**11% (9)**	14% (3)	11% (2)	15% (3)	5% (1)
CD4 count, cells/μL Median (IQR)*	**32 (8-58)**	24 (5-69)	31 (12-51)	32 (10-50)	32 (16-84)
Symptom duration in days Median (IQR)	**14 (7-16)**	14 (4-16)	14 (7-21)	9 (7-21)	8 (7-14)
Glasgow Coma Score < 15	**28% (22)**	29% (6)	28% (5)	25% (5)	30% (6)

% (n)					
CSF opening pressure cm H_20 Median (IQR)	**25 (16-36)**	22 (17-31)	22 (16-29)	32 (13-38)	27 (18-55)
CSF WCC cells/μL Median (IQR)*	**12 (5-64)**	10 (5-138)	15 (4-40)	15 (5-64)	13 (3-70)
CSF Fungal Burden log_{10}CFU/ml Median (IQR)*	**5.0 (3.7-5.8)**	4.9 (2.7-5.6)	5.2 (3.2-6.0)	5.3 (4.2-5.5)	5.0 (3.9-5.9)
Hemoglobin (g/dl) Median (IQR)	**11 (9.5-12.6)**	11.2 (9.5-12.5)	10.6 (9.5-12)	10.4(9.6-13.5)	11.7 (10.1-12.3)
Creatinine (umol/L) Median (IQR)	**63 (58-89)**	73 (59-103)	69 (59-89)	62 (57-75)	62 (55-95)

*5 patients were missing baseline CD4 counts, 5 patients were missing CSF white cell counts, and a single individual was mising baseline QCC. All other data were complete for all participants.

All patients were of black African ethnicity.

L-AmB: Liposomal amphotericin B.

Table 2: Primary and Key Secondary Outcomes

	All	**Control**	**Single Dose L-AmB**	**Two Doses L-AmB**	**Three Doses L-AmB**	*P-value*
Early Fungicidal Activity log_{10}CFU/ml/day (mean, 95% CI)	**-0.49 (-0.56, -0.41)** ***n=69****	-0.41 (-0.47, -0.36) *n=17*	-0.52 (-.71, -0.33) *n=16*	-0.47 (-0.6, -0.32) *n=18*	-0.54 (-0.76, -0.33) *n=18*	*0.64*
Mean difference in EFA versus control log_{10}CFU/ml/day (mean, 95% CI)	--	--	-0.11 (-0.29, 0.07)	-0.05 (-0.20, 0.10)	-0.13 (-0.35, 0.09)	†
2 week mortality, % (n)	**15% (12/79)**	10% (2/21)	11% (2/18)	15% (3/20)	25% (5/20)	*0.52*
10 week mortality, % (n)	**29% (23/79)**	29% (6/21)	22% (4/18)	15% (3/20)	50% (10/20)	*0.09*
Grade 3 AEs During Induction Therapy (days 1 – 14), % (n)						
Anaemia	**6% (5)**	0% (0)	11% (2)	15% (3)	0% (0)	*0.11*
Renal impairment	**5% (4)**	0% (0)	6% (1)	0% (0)	15% (3)	*0.10*

Hypokalemia	**1% (1)**	0% (0)	0% (0)	5% (1)	0% (0)	*0.39*
Grade 4 AEs During Induction Therapy (days 1 – 14), % (n)						
Anaemia	**1% (1)**	5% (1)	0% (0)	0% (0)	0% (0)	*0.42*
Renal impairment	**1% (1)**	0% (0)	0% (0)	0% (0)	5% (1)	*0.39*
Hypokalemia	**1% (1)**	5% (1)	0% (0)	0% (0)	0% (0)	*0.42*
Mean Change from Baseline to Day 14						
Haemoglobin g/dL (mean, 95% CI)	**0.9 (0.5, 1.4)**	1.2 (0.1, 2.3)	0.8 (-0.1, 1.7)	0.3 (-0.6, 1.3)	1.4 (0.5, 2.2)	*0.39*
Creatinine % (mean, 95% CI)	**14% (3, 24%)**	17% (-9, 43%)	13% (-9, 35%)	24% (6, 42%)	-2% (-22, 18%)	*0.29*

*Individuals who die prior to the day 3 LP or who were culture negative at baseline do not have EFA value. Overall 5 patients died prior to follow-up LP (1 control, 1 single dose, 1 two dose, 2 three dose) and 5 patients had negative baseline cultures (3 controls, 1 single dose, 1 2 dose).

†All three study arms were non inferior to control at the pre-defined non-inferiority margin of 0.2 $\log_{10}$CFU/ml/day. The respective 99% confidence intervals for the difference in mean EFA between single dose and control was -0.11 (99% CI -0.35 to 0.14) $\log_{10}$CFU/mL/day; between two doses and control -0.05 (99% CI -0.26 to 0.16) log10CFU/mL/day; and between three doses and control was -0.13 (95% CI -0.42 to 0.17) $\log_{10}$CFU/mL/day. Using this more stringent cut-off, all three study arms remained non inferior to control at the pre-defined non-inferiority margin of 0.2 $\log_{10}$CFU/ml/day.

L-AmB: Liposomal amphotericin B.

Table 3: Adverse Events and Readmissions During 10-week Follow-up

	All	**Control**	**Single Dose L-AmB**	**Two Doses L-AmB**	**Three Doses L-AmB**	*P-value*
Overall						
All AEs	**88***	19	29	14	26	*0.50*
Grade 3 AEs	**45**	7	18	9	11	-
Elevated creatinine		0	2	1	3	
Hypokalaemia		1	0	1	0	
Hypomannesaemia		1	0	0	0	
Hyponatraemia		2	4	1	0	
Elevated ALT		0	2	1	2	
Anaemia		0	2	1	0	
Neutropenia		1	2	2	0	
Prolonged initial hospitalisation		2	3	1	5	
Persistently raised ICP		0	1	1	0	
Other		0	Co-trimoxazole	0	Pneumonia	

			allergy			
			Confusion			
Grade 4 AEs	**20**	6	7	2	5	-
Elevated creatinine		1	1	0	1	
Hypokalaemia		1	0	0	0	
Hyponatraemia		2	0	0	1	
Hypernatraemia		0	1	0	0	
Elevated ALT		0	0	1	0	
Anaemia		1	0	0	0	
Neutropaenia		0	1	1	0	
Prolonged initial hospitalisation		0	1	0	1	
Recurrence of CM symptoms		1	1	0	2	
Other		0	Recurrent seizures Persistently raised ICP	0	0	
Grade 5 AEs (Deaths)	**23**	6	4	3	10	-

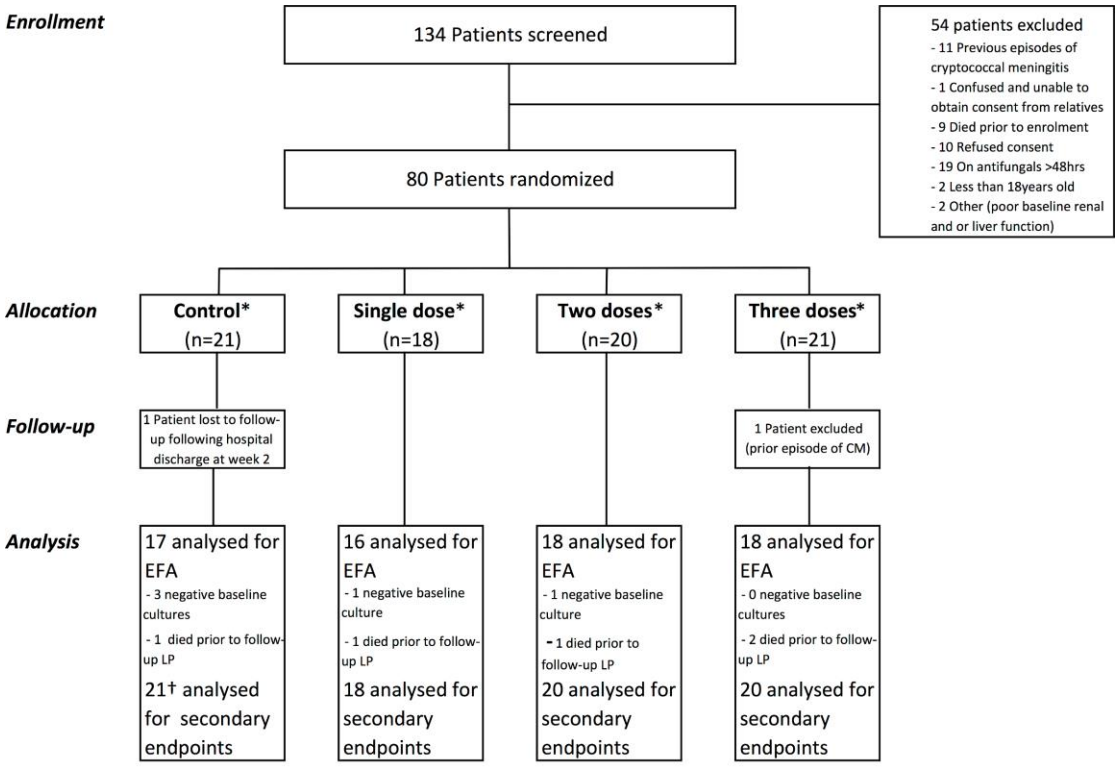
Adverse Events Related to Liposomal Amphotericin B Therapy†						
Grade 3 AEs	**10**	3	2	2	3	-
Grade 4 AEs	**2**	2	0	0	0	-
Readmissions and Immune Reconstitution Inflammatory Syndrome						
Readmissions	**11**	4	4	0	3	0.48
Possible IRIS	**5**	1	1	0	3	

*47 patients had at least 1 AE: 2 patients had 5 AEs, 2 patients had 4 AEs, 5 patients had 3 AEs, 7 patients had 2 AEs, 6 patients had 1 AE, 33 had no AEs. 28 patients had grade 3 AEs: 4 in the control arm, 11 in the single dose arm, 6 in the two dose arm, and 7 in the three dose arm. 15 patients had grade 4 AEs: 5 in the control arm, 5 in the single dose arm, 2 in the two dose arm, and 3 in the three dose arm.

†Related includes all AEs classified as possibly, probably, or definitely related to study drug.

L-AmB: Liposomal amphotericin B.

Figure 1.



*All patients received their allocated intervention.No patients lost to follow-up during initial two-week follow-up.
†The single patient lost to follow-up had full follow-up data until hopsital discharge at 2 weeks which were used in toxicity and mortality analyses.

Figure 2.