How applicable is the single-dose AMBITION regimen for HIV-associated cryptococcal meningitis to high-income settings?

Thomas S Harrison[1,2,3], David S Lawrence[4,5], Henry C Mwandumba[6,7,8], David R Boulware[9,10], Mina C Hosseinipour[11,12], Olivier Lortholary[13,14], Graeme Meintjes[15], Mosepele Mosepele[5,16], Joseph N Jarvis[4,5]†.

[1]Institute of Infection and Immunity, St George's University London, London, UK

[2]Clinical Academic Group in Infection and Immunity, St George's University Hospitals NHS Foundation Trust, London, UK

[3]MRC Centre for Medical Mycology, University of Exeter, Exeter, UK

[4]Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK

[5]Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana

[6]Liverpool School of Tropical Medicine, Liverpool, UK

[7]Malawi-Liverpool-Wellcome Clinical Research Programme, Blantyre, Malawi

[8]Department of Medicine, Kamuzu University of Health Sciences, Blantyre, Malawi

[9]Infectious Diseases Institute, College of Health Sciences, Makerere University, Kampala, Uganda

[10]University of Minnesota, Minneapolis, Minnesota, USA

[11]Lilongwe Medical Relief Trust (UNC Project), Lilongwe, Malawi

[12]Department of Medicine, University of North Carolina, Chapel Hill, North Carolina, USA

[13] Institut Pasteur, National Center for Scientific Research, Molecular Mycology Unit and National Reference Center for Invasive Mycoses and Antifungals, Unités Mixtes de Recherche 2000

[14] Université de Paris-Cité, Necker Pasteur Center for Infectious Diseases and Tropical Medicine, Hôpital Necker Enfants Malades, Assistance Publique–Hôpitaux de Paris, IHU Imagine

[15] The Wellcome Centre for Infectious Diseases Research in Africa, Institute of Infectious Disease and Molecular Medicine and the Department of Medicine, University of Cape Town, Cape Town, South Africa

[16]Department of Internal Medicine, University of Botswana, Gaborone, Botswana

†Corresponding Author: Professor Joseph N Jarvis. Botswana Harvard AIDS Institute Partnership, Private Bag BO320, Gaborone, Botswana. Email: joseph.jarvis@lshtm.ac.uk.

Alternative Corresponding Author: Professor Thomas S Harrison. Institute for Infection and Immunity, St. George's University of London, Cranmer Terrace, London SW17 0RE. tharriso@sgul.ac.uk.

Brief Summary (40 words): The AMBITION-cm trial showed that a single high dose of liposomal amphotericin B, given with oral fluconazole and flucytosine, is an effective treatment for HIV-associated cryptococcal meningitis. We argue that this is an appropriate treatment option for high-income country settings.

Running Title (40 characters): Cryptococcal meningitis treatment

Abstract

The AMBITION-cm phase III randomized controlled trial, conducted in east and southern Africa, showed that a single high dose (10mg/kg) of liposomal amphotericin B, given with an optimized oral backbone of fluconazole and flucytosine, was non-inferior to the World Health Organization (WHO)-recommended regimen of seven days of amphotericin B deoxycholate plus flucytosine for treatment of HIV-associated cryptococcal meningitis, and has been incorporated into updated WHO treatment guidelines. We believe the trial findings also have important implications for the treatment of HIV-associated cryptococcal meningitis in high-income settings. We advance the arguments, supported by evidence where available, that the AMBITION-cm study regimen is likely to be (i) as fungicidal as the currently recommended 14-day liposomal amphotericin based treatments, (ii) better tolerated with fewer adverse effects, and (iii) confer significant economic and practical benefits, therefore should be included as a treatment option in guidance for HIV-associated cryptococcal treatment in high-income country settings.

Keywords: Cryptococcal meningitis; HIV; Amphotericin B; Fluconazole; Flucytosine

Viewpoint

HIV-associated cryptococcal meningitis remains a significant driver of AIDS-related mortality, causing about 15% of all AIDS-related deaths. The greatest burden of disease is found in sub-Saharan Africa[1], primarily due to the persistent burden of advanced HIV disease despite widespread access to antiretroviral therapy[2]. Given the distribution of global disease burden, the vast majority of recent clinical research guiding cryptococcal management in people living with HIV has been generated in low- and middle-income countries (LMICs)[3].

Although the disease burden has lessened in high-income countries, HIV-related cryptococcosis still occurs and mortality is still substantial[4], with an estimated 7400 cases and 2000 deaths annually across Europe and North America[1]. The objective of this viewpoint is to present an overview of the findings of the recent AMBITION trial[5] and discuss their applicability to high-income settings. Data generated in LMICs have historically been overlooked in the development of high-income country guidelines. It is challenging to compare contexts, particularly when control regimens used in LMIC trials differ from the high-income country standard of care, and the ability to monitor and manage other HIV- and treatment-related complications vary, such that simple comparison of reported mortalities in high-income and LMIC studies is inappropriate. Nevertheless, we will argue that recent, high-quality data for novel treatment approaches from large, multi-site randomized controlled trials provide critical insights into drug action and toxicity and options for treatment that are universally applicable; and should therefore be considered in high-income settings. The viewpoint covers only HIV-associated cryptococcal meningitis, and treatment of other risk groups requires specific studies.

Short-course amphotericin-based treatment for cryptococcal meningitis

A program of clinical trials across sub-Saharan Africa was initiated in 2004. The aim was to develop and test new antifungal regimens, based on current drugs, that would be safer and more sustainable than the international standard of 2 weeks of amphotericin plus flucytosine, established by the ACTG trial of van der Horst and colleagues[6], and also more effective than widely-available and used fluconazole monotherapy[7]. Based on a series of promising phase 2 studies[8-10], the ACTA trial recruited 722 people with HIV-related cryptococcal meningitis who were randomized to one of five arms: oral combination therapy with high dose fluconazole (1200 mg/day) plus flucytosine, 1 week of amphotericin deoxycholate (1 mg/kg/day) with either fluconazole (1200 mg/day) or flucytosine (100 mg/kg/day), or the established standard of 2 weeks of amphotericin deoxycholate, again with either fluconazole or flucytosine[11]. One-week of amphotericin was non-inferior to treatment for 2 weeks in terms of all-cause mortality through 10 weeks (Hazard Ratio 0.89; 95%CI, 0.66-1.21). In addition, those randomized to 1-week amphotericin plus flucytosine experienced the lowest 10-week mortality when compared with all other regimens, including 14 days of the same amphotericin plus flucytosine therapy (24% vs 38%, Hazard Ratio 0.56; 95%CI, 0.35-0.91). The results reflected an optimal balance between fungicidal activity and toxicity with the 1-week regimen. The shorter course of amphotericin significantly reduced amphotericin-related toxicities, particularly anemia and renal impairment, without a reduction in fungicidal activity, probably due to the long half-life of amphotericin. Flucytosine was the best partner drug with amphotericin B, associated with reduced mortality and enhanced fungicidal activity. The oral combination arm had fewest side

effects and was the second-best performing regimen overall, despite less rapid fungicidal activity. As a result, in 2018, the WHO recommended 1-week amphotericin plus flucytosine followed by seven days of fluconazole 1200 mg/day as first-line therapy [12]. In addition, the oral combination arm was recommended if amphotericin was unavailable.

Subsequently, advocacy efforts and support from Unitaid and partners has led to increasing availability of more affordable generic flucytosine, and results of implementation of the 1-week amphotericin plus flucytosine regimen have mirrored the mortality reduction seen in ACTA, with in-hospital mortality in South Africa reduced from 37% (based largely on the prior standard there of 2-weeks amphotericin plus fluconazole) to 24% [13].

However, even a 1-week course of amphotericin deoxycholate has significant toxicities[11], which prompted work to determine if novel, short-course treatment with liposomal amphotericin (AmBisome, Gilead Sciences, Foster City, CA) could be clinically efficacious, safe, and cost-effective. Proof-of-concept for a single, high-dose of AmBisome was established in visceral leishmaniasis[14]. In addition, AmBisome has a very long-half life in the brain tissue in animal models[15].

Single high-dose liposomal amphotericin-based therapy

The AMBITION Phase II trial was a multi-site, randomized controlled-trial with the objective of finding the optimal high-dose, short-course AmBisome dosing strategy for cryptococcal meningitis[16]. Early fungicidal activity (EFA) was the primary endpoint. While EFA, the rate of

fall in cryptococcal Colony Forming Units per ml CSF per day derived from serial lumbar punctures and quantitative CSF cultures, is not a perfect surrogate and does not capture issues of toxicity, it is independently associated with clinical outcome and is a quantitative metric of antifungal activity at the site of infection in humans[17, 18]. The trial had four arms:

1) AmBisome 10mg/kg/day on day 1 (single dose),

2) AmBisome 10 mg/kg/day on day 1 and 5mg/kg/day on day 3 (2 doses),

3) AmBisome 10mg/kg/day on day 1 and 5mg/kg/day on days 3 and 7 (3 doses)

4) AmBisome 3mg/kg/day for 14 days (control).

All patients also received fluconazole 1200 mg/day for 14 days. Eighty participants were enrolled before the study was stopped on recommendation of the independent data monitoring committee. The antifungal activity was similar across the three short-course, high-dose AmBisome arms which were all non-inferior to the control 14-day regimen (EFA of single dose -0.52 $\log_{10}$CFU/mL /day (SD 0.35), vs control -0.41 $\log_{10}$CFU/mL /day (SD 0.11)), with no suggestion of a dose response with additional doses, and no safety concerns[18]. The single dose regimen was therefore taken forward to phase III.

At this time the ACTA results became available showing the superiority of flucytosine as a partner drug with amphotericin. There were however concerns that if we simply switched to a single-dose AmBisome plus flucytosine combination, then towards the end of the induction period low levels of AmBisome could effectively result in flucytosine monotherapy, risking the development of flucytosine resistance given its low barrier to resistance. Adding single-dose AmBisome to the optimized oral backbone of high-dose fluconazole plus flucytosine, which

even on its own performed well in ACTA, would protect flucytosine, while giving a needed amphotericin-related fungicidal boost to the oral regimen. In addition, prior phase 2 studies[10] supported earlier animal model work[19] that when higher, more effective doses of fluconazole are used, the triple combination of amphotericin, flucytosine and fluconazole is associated with the most rapid fungicidal activity (in contrast to earlier results using lower fluconazole doses[20]). The intervention thus brought together the strength of the oral combination arm observed in ACTA and added the single, high-dose of AmBisome shown in the AMBITION Phase II trial to be safe, and the most practical and efficient means to deliver liposomal amphotericin.

The AMBITION-cm trial

The AMBITION phase III trial was a non-inferiority randomized controlled trial of a single, high-dose of AmBisome given with 14 days of flucytosine and fluconazole in comparison with the WHO standard of care as previously defined: 7 days amphotericin deoxycholate plus flucytosine, followed by 7 days of fluconazole[5, 12]. The trial recruited 844 participants from eight hospitals in five countries: Botswana, Malawi, South Africa, Uganda, and Zimbabwe. 814 participants were included in intention-to-treat analysis, 407 in each arm, and no participants were lost to follow-up. At enrolment the median CD4 count was 27 cells/mm^3, and 28.5% of participants had abnormal mental status, indicating severe disease. Ten-week mortality was 24.8% (101/407; 95%CI, 20.7-29.3%) in the AmBisome arm and 28.7% (117/407; 95%CI, 24.4-33.4%) in the control arm. The absolute difference in 10-week mortality risk between the AmBisome arm and control was -3.9% with an upper limit one-sided 95% confidence interval of 1.2%, well below the pre-specified 10% non-inferiority margin. When adjusting for factors

associated with mortality, the AmBisome regimen was found to be just superior at 10 weeks. The mean rate of fungal clearance from the CSF was -0.40 $\log_{10}$ CFU/ml/day in the AmBisome group and -0.42 $\log_{10}$ CFU/ml/day in the control group with no significant difference between arms[5].

In addition, the AmBisome regimen was associated with significantly fewer adverse events including anemia, thrombophlebitis and electrolyte abnormalities. Grade 3 or 4 anemia developed in 13.3% of participants on AmBisome compared to 39.1% in the control group (p<.001)[5]. The mean decrease in hemoglobin over the first week was 0.3g/dL for AmBisome group and 1.9g/dL for control (p<.001); 7.6% of participants on AmBisome received a blood transfusion, compared to 18.0% for control. The mean increase in creatinine from baseline to day 7 was 20.2% on AmBisome group and 49.7% for control (p<.001). Thrombophlebitis requiring antibiotic therapy occurred in 1.9% of participants on AmBisome and 6.7% for the control group (p=.001). There was a low frequency of grade 4 thrombocytopenia, neutropenia, and elevated alanine aminotransferase in both AmBisome and control groups. The results have prompted the WHO to update their guidance to recommend the single 10 mg/kg liposomal amphotericin-based regimen as the preferred regimen[21], and implementation efforts in LMIC are already underway supported by Unitaid, CDC, Clinton Health Access Initiative, Médecins Sans Frontières, and others. Gilead have re-affirmed their commitment to not-for-profit LMIC pricing for AmBisome for cryptococcal meningitis [22].

Use of the AMBITION regimen in high-income settings

What about in high-income country settings? Are patients living with HIV in high-income countries now actually at risk of being "left behind", with unnecessarily long and toxic 2-week courses of daily liposomal amphotericin, or even amphotericin deoxycholate? The recommended first-line induction regimen in high-income settings is liposomal amphotericin at 3-4mg/kg plus flucytosine 100mg/kg/day for 14 days, a recommendation consistent across the Infectious Diseases Society of America, British HIV Association, and European AIDS Clinical Society[23-26]. These guidelines are based on the van der Horst trial of 2 weeks amphotericin deoxycholate plus flucytosine[6], and a subsequent transition over time from conventional amphotericin deoxycholate towards the liposomal formulation, based on the study of Hamill and colleagues comparing the liposomal and deoxycholate formulations, given as monotherapy[27]. In fact to date, no randomized controlled trials have tested the 2-week liposomal amphotericin plus flucytosine treatment regimen recommended in these guidelines.

Fungicidal activity

In terms of fungicidal activity, the AMBITION phase 3 trial demonstrated that the EFA of the single, high-dose AmBisome regimen was no different to that achieved with seven days of amphotericin deoxycholate-based treatment, and the AMBITION phase 2 data show that the single, high-dose regimen is non-inferior and may be marginally superior in EFA to standard daily AmBisome dosing for 14 days. Although the numbers of patients treated was small, across the 3 intermittent dosing arms the EFA was more rapid than control daily dosing (-0.52, -0.47, and -0.54 for 1, 2, and 3 doses respectively, compared with -0.41 $\log_{10}$CFU/mL /day for daily [17]), perhaps due to more rapid loading of brain compartments with the initial 10 mg/kg

dose on day 1. In addition, the effect of the single dose regimen is durable. In the AMBITION trial, no culture positive relapses occurred within the 10-week follow up period in the 407 participants treated [5]. This, despite the fact that the trial included many patients with severe disease and heavy organism load and participants with, in general, higher fungal burdens than usually seen in high-income settings [6, 28].

Hamill et al is the only trial providing data in high-income settings on the sterilizing effect of daily AmBisome for 14 days[27]. This was a three-arm comparison of amphotericin deoxycholate 0.7 mg/kg, AmBisome 3mg/kg, and AmBisome 6mg/kg in North America, with the aim of administering a full, uninterrupted 14-day course. A minimum of 11 days was required and in participants with delayed improvement treatment was continued for up to 21 days. The primary outcome was CSF sterility at two weeks. Eighty six participants were randomized to 3mg/kg AmBisome, 94 to 6 mg/kg/d AmBisome, and 87 to amphotericin deoxycholate. Of note, a number of participants did not complete the study for reasons including adverse events, lack of efficacy, loss to follow-up, and physician decision. Repeat CSF cultures at 14 days were negative in 58% of evaluable patients (positive baseline culture, and at least one follow-up culture) who received AmBisome 3mg/kg, 48% with AmBisome 6 mg/kg, and 48% with amphotericin deoxycholate. By 10 weeks, the percentage evaluable with negative CSF cultures was 60%, 71%, and 79%, respectively[28]. This compares with 77% (255/332) CSF culture conversion at 2 weeks for the single dose AmBisome regimen in AMBITION, where all survivors had a day 14 LP[27].

While there is no large randomized trial comparison, it is plausible that daily 3-4 mg/kg/day AmBisome is less active in terms of EFA than either amphotericin deoxycholate at 1 mg/kg/d, or

the AMBITION single, high-dose regimen, with no data to suggest that 3-4 mg/kg/d AmBisome would be more fungicidal. We would contend that overall the evidence suggests that the AMBITION *triple* therapy regimen would have at least equivalent fungicidal activity as 14 days of daily 3-4 mg/kg Ambisome plus flucytosine, and should not be regarded as a compromise regimen, of interest only to resource-limited settings, in terms of antifungal effect (Figure 1).

Clinical efficacy

It is difficult to generalize that AmBisome is equally effective but safer than amphotericin deoxycholate: it depends on the dose of both drugs. Hamill et al. used amphotericin deoxycholate at 0.7 mg/kg/day, and we know from LMIC data that 1 mg/kg/day has greater antifungal activity[29]. Based on the Hamill study comparing with 0.7 mg/kg/d deoxycholate, the FDA only approved the 6 mg/kg/day dose of AmBisome[31]. In the FDA analysis, combined clinical success and culture conversion by 10 weeks in those with a positive baseline culture was 37%, 49%, and 53%, for AmBisome 3 mg/kg/d, AmBisome 6 mg/kg/d, and amphotericin deoxycholate, respectively[31]. But with 6 mg/kg/d, adverse events are comparable to those with amphotericin deoxycholate[28], and costs are increased substantially.

Mortality in the van der Horst trial was 5.5% at 2 weeks and 3.9% between 2 and 10 weeks, and it is sometimes assumed therefore that 10-week mortality was 9.4%. However, the trial was conducted in 2 stages, and only participants responding to treatment at 2 weeks were continued in the trial and re-randomized to fluconazole vs itraconazole for consolidation treatment[6]. Of 381 participants initially randomized, 21 died in the first 2 weeks and, in the second step, 12 between 2 and 10 weeks. But of 360 survivors at 2 weeks, 54 (who were not stable or had not improved and were likely to have poor outcomes) were not followed up. 7

were known to have died but outcomes for the other 47 are unknown (van der Horst, personal communication in 2010). Thus, 10-week mortality was actually up to 23% (87/361), depending how many of these participants died before 10 weeks. In addition, there was no next-of-kin consent and the exclusion criteria were more extensive than in ACTA and AMBITION, where patients were not excluded on the basis of markers of severity and next-of-kin could consent for confused and reduced conscious level patients (in AMBITION, 10-week mortality with the Ambisome regimen for those with GCS 15 was 16.8%). Cohort data from high-income settings report 70-90 day mortalities of 15-26%[4][30-33], overall lower but not so different from our latest trial results, and probably driven by earlier presentation and greater ability to monitor and manage other HIV- and treatment-related complications, rather than superiority of antifungal regimen. Thus, we would argue that the AMBITION regimen should not be ruled out for high-income settings on the basis of mortality comparisons.

Safety

In terms of safety, the data suggest that the single AmBisome dose regimen has advantages over current guidance. In the Hamill trial, 23.3% of those on 3 mg/kg/d and 41% of those on 6 mg/kg/d Ambisome developed a hemoglobin $\leq$8 g/dL[27]. In AMBITION the cut off for a grade 3 anemia was <9.0 g/dL in women and <8.5 g/dL in men, somewhat higher, yet only 13.3% of those on the single dose regimen developed this level of anemia[5]. In prior studies, both anemia and rises in creatinine have been associated with increased mortality[34]. As described above, the AMBITION regimen was similarly "clean" in terms of renal impairment, hypokalemia, and, unsurprisingly, given the need for just one infusion, line infections – a source of serious bacterial sepsis[35]. While consistent close monitoring and management of side effects may be

more feasible in high-income than LMIC settings, this does not eliminate the occurrence of serious side effects, nor completely avoid the associated morbidity and mortality. Patients in high-income settings will also benefit from a safer regimen.

Cost and acceptability

Finally, on cost, convenience, and patient and provider preference, the AMBITION regimen has clear advantages. In a formal health economic analysis, the AMBITION regimen is only marginally more costly than 1-week of amphotericin deoxycholate plus flucytosine (Lawrence D, submitted). Further comparisons are underway, but there will be very significant cost savings with the AMBITION regimen compared to 2-week liposomal amphotericin-based regimens – driven by the possibility of shorter hospitalization, and a 5-fold reduction in AmBisome drug requirement (10 mg/kg total vs 49 mg/kg total, for a 14 day course at 3.5 mg/kg/day). A retrospective analysis of 24,151 patients with HIV-associated cryptococcal meningitis who were treated in the USA between 1997 and 2009, calculated an average hospitalization cost of $15,708 per patient[36], since when costs have increased significantly[37]. In addition, a social science sub-study of the AMBITION trial, points to a clear preference on the part of participants and health care providers for the single dose regimen (Lawrence D, personal communication). Given their vulnerable status, and ongoing nosocomial infection risks, patients in high-income countries with less severe disease may also welcome and benefit from the simplified delivery of treatment and possibility of earlier discharge with the AMBITION regimen. The median duration of hospitalization in successful implementation of the 1-week amphotericin deoxycholate regimen in South Africa was 10 days [14], and discharge before day 14 could be conditional on close outpatient follow-up.

Conclusions

From high-income countries, there are no recent controlled trials of HIV-associated cryptococcal meningitis, with a total of 13 trials published between 1990 and 2010, which recruited a total of 1623 patients in high-income settings from 1987 to 2007[3]. This compares with 4275 patients recruited in LMICs up to and including the recent AMBITION trial[3]. No randomized controlled trial data support current European and U.S. treatment guidance, and new high-income country only trials will be challenging due to the dispersed case burden. Future trials that incorporate new antifungal agents should include recruitment in high-income settings with local standard of care comparisons at those sites. Meanwhile, for high-income countries, careful evaluation of evidence from LMIC is warranted, just as physicians in LMIC settings routinely adapt evidence from high-income countries to their context.

In conclusion, there are limited comparable data from high-income countries to clearly compare the clinical, microbiological and safety outcomes observed in the AMBITION trial with those observed in high-income settings where patients are managed with 2 weeks of liposomal amphotericin plus flucytosine. The EFA, the high rates of CSF sterility at two weeks, and the absence of relapse cases observed in the AMBITION trial indicate that the AMBITION antifungal combination is extremely effective at clearing *Cryptococcus* from the CSF. In addition, the shorter duration of intravenous treatment and the low rates of drug-related toxicity compared to clinical trial data of prolonged courses of liposomal amphotericin indicate that this is a safe and convenient treatment regimen. We would argue that the AMBITION regimen should be

included as a treatment option in guidance for HIV-associated cryptococcal treatment in high-income country settings. As with any new treatment, context-specific algorithms could enable optimal, safe delivery, and ongoing monitoring and evaluation of outcomes will be important.

Funding

Funding was provided by the National Institute for Health Research (NIHR) through a Global Health Research Professorship (RP-2017-08-ST2-012, to Dr. Jarvis) with aid from the U.K. government to support global health research.

Conflict of interest

Dr. Harrison reports grants, personal fees and Investigator award (to institution) from Gilead Sciences, personal fees from Pfizer, personal fees from F2G, and provision of drug for Ambition-CM trial from Gilead Sciences, outside the submitted work; Dr. Boulware reports grants research support and fees from Gilead, outside the submitted work; Dr. Lortholary reports grants and personal fees from Gilead Sciences, including <1000 USD honoraria, outside the submitted work; Dr. Jarvis reports grants and personal speaker fees from Gilead Sciences, outside the submitted, grant funding to institution (completed) from EDCTP and to institution (ongoing) from CDC, participation on a Data Safety Monitoring Board or Advisory Board for HARVEST, ARTIST, ASTRO, CASTLE, and ACACIA Trials. Dr. Mwandumba reports support for research not related to the present manuscript from UKRI, Bill and Melinda Gates Foundation, and National Institutes of Health; interview and advisory roles with Wellcome Career

Development Award Panel, and roles as Council Member for International Society for Infectious Diseases and International Union of Immunological Societies, and is an Executive Committee Member of the Federation of African Immunological Societies. Dr. Meintjes reports ZAR 12,000 for delivering an educational lecture from Gilead. All other authors have no conflicts of interest to declare.

References:

1. 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**; 17(8): 873-81.
2. Tenforde MW, Mokomane M, Leeme T, et al. Advanced Human Immunodeficiency Virus Disease in Botswana Following Successful Antiretroviral Therapy Rollout: Incidence of and Temporal Trends in Cryptococcal Meningitis. Clin Infect Dis **2017**; 65(5): 779-86.
3. Lawrence DS, Leeme T, Mosepele M, Harrison TS, Seeley J, Jarvis JN. Equity in clinical trials for HIV-associated cryptococcal meningitis: A systematic review of global representation and inclusion of patients and researchers. PLoS Negl Trop Dis **2021**; 15(5): e0009376.
4. Hevey MA, Presti RM, O'Halloran JA, et al. Mortality After Cryptococcal Infection in the Modern Antiretroviral Therapy Era. J Acquir Immune Defic Syndr **2019**; 82(1): 81-7.
5. Jarvis JN, Lawrence DS, Meya DB, et al. Single-Dose Liposomal Amphotericin B Treatment for Cryptococcal Meningitis. N Engl J Med **2022**; 386(12): 1109-20.
6. van der Horst CM, Saag MS, Cloud GA, et al. Treatment of cryptococcal meningitis associated with the acquired immunodeficiency syndrome. National Institute of Allergy and Infectious Diseases Mycoses Study Group and AIDS Clinical Trials Group. The New England journal of medicine **1997**; 337(1): 15-21.
7. Rothe C, Sloan DJ, Goodson P, et al. A prospective longitudinal study of the clinical outcomes from cryptococcal meningitis following treatment induction with 800 mg oral fluconazole in Blantyre, Malawi. PLoS One **2013**; 8(6): e67311.
8. 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.
9. Nussbaum JC, Jackson A, Namarika D, et al. Combination flucytosine and high-dose fluconazole compared with fluconazole monotherapy for the treatment of cryptococcal meningitis: a randomized trial in Malawi. Clin Infect Dis **2010**; 50(3): 338-44.
10. Jackson AT, Nussbaum JC, Phulusa J, et al. A phase II randomized controlled trial adding oral flucytosine to high-dose fluconazole, with short-course amphotericin B, for cryptococcal meningitis. AIDS **2012**; 26(11): 1363-70.
11. Molloy SF, Kanyama C, Heyderman RS, et al. Antifungal Combinations for Treatment of Cryptococcal Meningitis in Africa. N Engl J Med **2018**; 378(11): 1004-17.
12. WHO. Guidelines for the diagnosis, prevention, and management of cryptococcal disease in HIV-infected adults, adolescents and children. Available at: https://apps.who.int/iris/bitstream/handle/10665/260399/9789241550277-eng.pdf. Accessed 25 Oct.
13. Mashau RC, Nel J, Meiring ST, et al. Outcomes of flucytosine-containing combination treatment for cryptococcal meningitis in a South African national access programme. The Lancet infectious diseases **2022**: In Press.

14. Sundar S, Chakravarty J, Agarwal D, Rai M, Murray HW. Single-dose liposomal amphotericin B for visceral leishmaniasis in India. N Engl J Med **2010**; 362(6): 504-12.
15. Lestner J, McEntee L, Johnson A, et al. Experimental Models of Short Courses of Liposomal Amphotericin B for Induction Therapy for Cryptococcal Meningitis. Antimicrob Agents Chemother **2017**; 61(6): e00090-17.
16. 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.
17. Pullen MF, Hullsiek KH, Rhein J, et al. Cerebrospinal Fluid Early Fungicidal Activity as a Surrogate Endpoint for Cryptococcal Meningitis Survival in Clinical Trials. Clin Infect Dis **2020**; 71(7): e45-e9.
18. Jarvis JN, Leeme TB, Molefi M, et al. Short-course High-dose Liposomal Amphotericin B for Human Immunodeficiency Virus-associated Cryptococcal Meningitis: A Phase 2 Randomized Controlled Trial. Clin Infect Dis **2019**; 68(3): 393-401.
19. Diamond DM, Bauer M, Daniel BE, et al. Amphotericin B colloidal dispersion combined with flucytosine with or without fluconazole for treatment of murine cryptococcal meningitis. Antimicrobial agents and chemotherapy **1998**; 42(3): 528-33.
20. 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.
21. World Health Organization. Rapid Advice: New guidelines from WHO recommend a simpler, safer treatment for cryptococcal disease in people living with HIV. Available at: https://www.who.int/news/item/20-04-2022-rapid-advice-new-guidelines-for-simpler-safer-treatment-for-cryptococcal-disease-in-plhiv. Accessed 20 April.
22. Gilead. Gilead Sciences Statement on Positive Phase 3 AMBITION Study Findings for the Treatment of HIV-Associated Cryptococcal Meningitis. Available at: https://www.gilead.com/news-and-press/company-statements/gilead-sciences-statement-on-positive-phase-3-ambition-study-findings-for-the-treatment-of-hiv-associated-cryptococcal-meningitis. Accessed 21 July.
23. 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: 291-322.
24. 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.
25. Ryom L, Cotter A, De Miguel R, et al. 2019 update of the European AIDS Clinical Society Guidelines for treatment of people living with HIV version 10.0. HIV medicine **2020**; 21(10): 617-24.
26. Panel on Guidelines for the Prevention and Treatment of Opportunistic Infections in Adults and Adolescents with HIV. Guidelines for the Prevention and Treatment of Opportunistic Infections in HIV-infected Adults and Adolescents: Recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America.

Available at: https://clinicalinfo.hiv.gov/en/guidelines/adult-and-adolescent-opportunistic-infection/cryptococcosis. Accessed 12 Jan.
27. 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.
28. Kambugu A, Meya DB, Rhein J, et al. Outcomes of cryptococcal meningitis in Uganda before and after the availability of highly active antiretroviral therapy. Clin Infect Dis **2008**; 46(11): 1694-701.
29. Bicanic T, Wood R, Meintjes G, et al. High-dose amphotericin B with flucytosine for the treatment of cryptococcal meningitis in HIV-infected patients: a randomized trial. Clin Infect Dis **2008**; 47(1): 123-30.
30. Brizendine KD, Baddley JW, Pappas PG. Predictors of mortality and differences in clinical features among patients with Cryptococcosis according to immune status. PLoS ONE **2013**; 8(3): e60431.
31. Lortholary O, Poizat G, Zeller V, et al. Long-term outcome of AIDS-associated cryptococcosis in the era of combination antiretroviral therapy. AIDS (London, England) **2006**; 20(17): 2183-91.
32. George IA, Spec A, Powderly WG, Santos CAQ. Comparative Epidemiology and Outcomes of Human Immunodeficiency virus (HIV), Non-HIV Non-transplant, and Solid Organ Transplant Associated Cryptococcosis: A Population-Based Study. Clin Infect Dis **2018**; 66(4): 608-11.
33. Robinson PA, Bauer M, Leal MA, et al. Early mycological treatment failure in AIDS-associated cryptococcal meningitis. Clin Infect Dis **1999**; 28(1): 82-92.
34. Tugume L, Morawski BM, Abassi M, et al. Prognostic implications of baseline anaemia and changes in haemoglobin concentrations with amphotericin B therapy for cryptococcal meningitis. HIV medicine **2017**; 18(1): 13-20.
35. Rajasingham R, Williams D, Meya DB, Meintjes G, Boulware DR, Scriven J. Nosocomial drug-resistant bacteremia in 2 cohorts with cryptococcal meningitis, Africa. Emerg Infect Dis **2014**; 20(4): 722-4.
36. Pyrgos V, Seitz AE, Steiner CA, Prevots DR, Williamson PR. Epidemiology of cryptococcal meningitis in the US: 1997-2009. PLoS One **2013**; 8(2): e56269.
37. Rajasingham R, Boulware DR. Reconsidering cryptococcal antigen screening in the U.S. among persons with CD4 <100 cells/mcL. Clin Infect Dis **2012**; 55(12): 1742-4.

Figure 1. Infographic