

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

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

16 Running Title (40 characters): Cryptococcal meningitis treatment

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1 **Abstract**

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

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

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1 **Viewpoint**

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

8
9 Although the disease burden has lessened in high-income countries, HIV-related cryptococcosis
10 still occurs and mortality is still substantial[4], with an estimated 7400 cases and 2000 deaths
11 annually across Europe and North America[1]. The objective of this viewpoint is to present an
12 overview of the findings of the recent AMBITION trial[5] and discuss their applicability to high-
13 income settings. Data generated in LMICs have historically been overlooked in the development
14 of high-income country guidelines. It is challenging to compare contexts, particularly when
15 control regimens used in LMIC trials differ from the high-income country standard of care, and
16 the ability to monitor and manage other HIV- and treatment-related complications vary, such
17 that simple comparison of reported mortalities in high-income and LMIC studies is
18 inappropriate. Nevertheless, we will argue that recent, high-quality data for novel treatment
19 approaches from large, multi-site randomized controlled trials provide critical insights into drug
20 action and toxicity and options for treatment that are universally applicable; and should
21 therefore be considered in high-income settings. The viewpoint covers only HIV-associated
22 cryptococcal meningitis, and treatment of other risk groups requires specific studies.

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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

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

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

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

18 19 ***Single high-dose liposomal amphotericin-based therapy***

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

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

- 5 1) AmBisome 10mg/kg/day on day 1 (single dose),
- 6 2) AmBisome 10 mg/kg/day on day 1 and 5mg/kg/day on day 3 (2 doses),
- 7 3) AmBisome 10mg/kg/day on day 1 and 5mg/kg/day on days 3 and 7 (3 doses)
- 8 4) AmBisome 3mg/kg/day for 14 days (control).

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

16
17 At this time the ACTA results became available showing the superiority of flucytosine as a
18 partner drug with amphotericin. There were however concerns that if we simply switched to a
19 single-dose AmBisome plus flucytosine combination, then towards the end of the induction
20 period low levels of AmBisome could effectively result in flucytosine monotherapy, risking the
21 development of flucytosine resistance given its low barrier to resistance. Adding single-dose
22 AmBisome to the optimized oral backbone of high-dose fluconazole plus flucytosine, which

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

9 10 ***The AMBITION-cm trial***

11 The AMBITION phase III trial was a non-inferiority randomized controlled trial of a single, high-
12 dose of AmBisome given with 14 days of flucytosine and fluconazole in comparison with the
13 WHO standard of care as previously defined: 7 days amphotericin deoxycholate plus
14 flucytosine, followed by 7 days of fluconazole[5, 12]. The trial recruited 844 participants from
15 eight hospitals in five countries: Botswana, Malawi, South Africa, Uganda, and Zimbabwe. 814
16 participants were included in intention-to-treat analysis, 407 in each arm, and no participants
17 were lost to follow-up. At enrolment the median CD4 count was 27 cells/mm³, and 28.5% of
18 participants had abnormal mental status, indicating severe disease. Ten-week mortality was
19 24.8% (101/407; 95%CI, 20.7-29.3%) in the AmBisome arm and 28.7% (117/407; 95%CI, 24.4-
20 33.4%) in the control arm. The absolute difference in 10-week mortality risk between the
21 AmBisome arm and control was -3.9% with an upper limit one-sided 95% confidence interval of
22 1.2%, well below the pre-specified 10% non-inferiority margin. When adjusting for factors

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

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

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1 ***Use of the AMBITION regimen in high-income settings***

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

14 ***Fungicidal activity***

15 In terms of fungicidal activity, the AMBITION phase 3 trial demonstrated that the EFA of the
16 single, high-dose AmBisome regimen was no different to that achieved with seven days of
17 amphotericin deoxycholate-based treatment, and the AMBITION phase 2 data show that the
18 single, high-dose regimen is non-inferior and may be marginally superior in EFA to standard
19 daily AmBisome dosing for 14 days. Although the numbers of patients treated was small,
20 across the 3 intermittent dosing arms the EFA was more rapid than control daily dosing (-0.52, -
21 0.47, and -0.54 for 1, 2, and 3 doses respectively, compared with -0.41 log₁₀CFU/mL /day for
22 daily [17]), perhaps due to more rapid loading of brain compartments with the initial 10 mg/kg

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

6 Hamill et al is the only trial providing data in high-income settings on the sterilizing effect of
7 daily AmBisome for 14 days[27]. This was a three-arm comparison of amphotericin
8 deoxycholate 0.7 mg/kg, AmBisome 3mg/kg, and AmBisome 6mg/kg in North America, with the
9 aim of administering a full, uninterrupted 14-day course. A minimum of 11 days was required
10 and in participants with delayed improvement treatment was continued for up to 21 days. The
11 primary outcome was CSF sterility at two weeks. Eighty six participants were randomized to
12 3mg/kg AmBisome, 94 to 6 mg/kg/d AmBisome, and 87 to amphotericin deoxycholate. Of note,
13 a number of participants did not complete the study for reasons including adverse events, lack
14 of efficacy, loss to follow-up, and physician decision. Repeat CSF cultures at 14 days were
15 negative in 58% of evaluable patients (positive baseline culture, and at least one follow-up
16 culture) who received AmBisome 3mg/kg, 48% with AmBisome 6 mg/kg, and 48% with
17 amphotericin deoxycholate. By 10 weeks, the percentage evaluable with negative CSF cultures
18 was 60%, 71%, and 79%, respectively[28]. This compares with 77% (255/332) CSF culture
19 conversion at 2 weeks for the single dose AmBisome regimen in AMBITION, where all survivors
20 had a day 14 LP[27].

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

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

6 *Clinical efficacy*

7 It is difficult to generalize that AmBisome is equally effective but safer than amphotericin
8 deoxycholate: it depends on the dose of both drugs. Hamill et al. used amphotericin
9 deoxycholate at 0.7 mg/kg/day, and we know from LMIC data that 1 mg/kg/day has greater
10 antifungal activity[29]. Based on the Hamill study comparing with 0.7 mg/kg/d deoxycholate,
11 the FDA only approved the 6 mg/kg/day dose of AmBisome[31]. In the FDA analysis, combined
12 clinical success and culture conversion by 10 weeks in those with a positive baseline culture was
13 37%, 49%, and 53%, for AmBisome 3 mg/kg/d, AmBisome 6 mg/kg/d, and amphotericin
14 deoxycholate, respectively[31]. But with 6 mg/kg/d, adverse events are comparable to those
15 with amphotericin deoxycholate[28], and costs are increased substantially.

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

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

13 *Safety*

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

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

4 *Cost and acceptability*

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

1

2 **Conclusions**

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

13

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

1 included as a treatment option in guidance for HIV-associated cryptococcal treatment in high-
2 income country settings. As with any new treatment, context-specific algorithms could enable
3 optimal, safe delivery, and ongoing monitoring and evaluation of outcomes will be important.
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10

11 **Conflict of interest**

12 Dr. Harrison reports grants, personal fees and Investigator award (to institution) from Gilead
13 Sciences, personal fees from Pfizer, personal fees from F2G, and provision of drug for Ambition-
14 CM trial from Gilead Sciences, outside the submitted work; Dr. Boulware reports grants
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1 Development Award Panel, and roles as Council Member for International Society for Infectious
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3 Member of the Federation of African Immunological Societies. Dr. Meintjes reports ZAR 12,000
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ACCEPTED MANUSCRIPT

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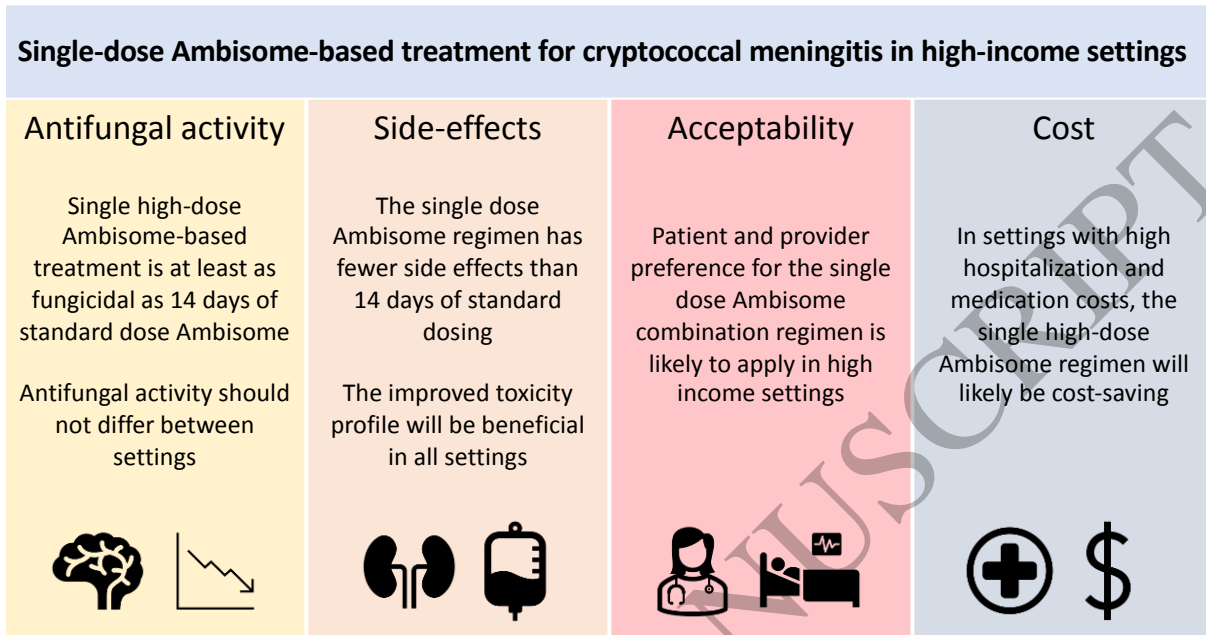
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1 **Figure 1.** Infographic



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ACCEPTED MANUSCRIPT