Adjunctive sertraline for HIV-associated cryptococcal meningitis: a randomised, placebo-controlled, double-blind phase 3 trial

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Summary

Background Identifying new antifungals for cryptococcal meningitis is a priority given the inadequacy of current therapy. Sertraline has previously shown in vitro and in vivo activity against cryptococcus. We aimed to assess the efficacy and cost-effectiveness of adjunctive sertraline in adults with HIV-associated cryptococcal meningitis compared with placebo.

Methods In this double-blind, randomised, placebo-controlled trial, we recruited HIV-positive adults with cryptococcal meningitis from two hospitals in Uganda. Participants were randomly assigned (1:1) to receive standard therapy with 7–14 days of intravenous amphotericin B (0·7–1·0 mg/kg per day) and oral fluconazole (starting at 800 mg/day) with either adjunctive sertraline or placebo. Sertraline was administered orally or via nasogastric tube at a dose of 400 mg/day for 2 weeks, followed by 200 mg/day for 12 weeks, then tapered off over 3 weeks. The primary endpoint was 18-week survival, analysed by intention-to-treat. This study is registered with ClinicalTrials.gov, number NCT01802385.

Findings Between March 9, 2015, and May 29, 2017, we screened 842 patients with suspected meningitis and enrolled 460 of a planned 550 participants, at which point the trial was stopped for futility. Three patients in the sertraline group and three patients in the placebo group were lost to follow-up and therefore discontinued before study end. At 18 weeks, 120 (52%) of 229 patients in the sertraline group and 106 (46%) of 231 patients in the placebo group had died (hazard ratio 1·21, 95% CI 0·93–1·57; p=0·15). The fungal clearance rate from cerebrospinal fluid was similar between groups (0·43 –$\log_{10}$ CFU/mL per day [95% CI 0·37–0·50] in the sertraline group *vs* 0·47 –$\log_{10}$ CFU/mL per day [0·40–0·54] in the placebo group; p=0·59), as was occurrence of grade 4 or 5 adverse events (72 [31%] of 229 *vs* 75 [32%] of 231; p=0·98), most of which were associated with amphotericin B toxicity.

Interpretation Sertraline did not reduce mortality and should not be used to treat patients with HIV-associated cryptococcal meningitis. The reasons for sertraline inactivity appear to be multifactorial and might be associated with insufficient duration of therapeutic sertraline concentrations.

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Introduction

Cryptococcal meningitis is one of the most common and deadly opportunistic infections in people with HIV, and is responsible for 15% of AIDS-related deaths.[1] Early mortality remains unacceptably high because of prohibitive costs, poor availability, and frequent toxicity associated with an insufficient armamentarium of effective antifungals. Given these challenges, more effective and less toxic antifungal regimens are urgently needed to improve outcomes. However, antifungal development is hindered by the high costs and time required to bring new drugs to market, and a reluctance for pharmaceutical companies to invest in drugs that offer little promise of financial return. As a result, there are few new anticryptococcal agents in the development pipeline.

Given the inherent challenges involved in de-novo antifungal development, drug repurposing is becoming an attractive approach for new antifungal discovery. This approach reduces the time required from bench to bedside, especially when it involves a new indication for an existing and widely used marketed drug. The selective serotonin reuptake inhibitor antidepressant sertraline provides an archetypal example of such repurposing for a potential role in the treatment of cryptococcal meningitis.

Sertraline has been shown in several studies to have potent in-vitro fungicidal activity against *Cryptococcus neoformans* that is synergistic to fluconazole.[2–7] Sertraline has also been effective in experimentally infected mice, in which the inhibitory effect of sertraline was particularly potent in the brain of cryptococcus-infected mice, with efficacy similar to fluconazole.[3,5] The most potent

Research in context

Evidence before this study

Treatment for cryptococcal meningitis is associated with unacceptably high mortality rates, and new therapeutic agents are urgently needed. Sertraline has potential clinical use for cryptococcus infection. We searched PubMed for articles in English from Jan 1, 1990, to Oct 31, 2018, to evaluate the existing evidence for sertraline as an antifungal against cryptococcus. Search terms included "sertraline" combined with "antifungal", "cryptococcus", or "cryptococcal meningitis". We identified five high-quality studies that showed potent in-vitro activity of sertraline against *Cryptococcus neoformans*, with synergistic effects in combination with fluconazole. Sertraline was also effective in reducing fungal burden in brain tissue in two studies that used mice models of cryptococcal infection. Finally, two human trials, including our 2015 pilot study, have investigated the effect of adjunctive sertraline at doses of 200–400 mg/day on fungal clearance in cerebral spinal fluid. In these studies, adjunctive sertraline was well tolerated and appeared to improve fungal clearance when added to standard amphotericin-B-based therapy.

Added value of this study

In this double-blind, randomised placebo-controlled trial, we evaluated the effect of adjunctive sertraline on the survival of patients with HIV-associated cryptococcal meningitis. This trial is the largest human study to date evaluating the efficacy of sertraline as an antifungal against cryptococcus in a real-world setting. Adjunctive sertraline at doses of 200–400 mg/day neither improved survival nor resulted in more rapid fungal clearance, and the trial was stopped for futility after enrolling 460 of a planned 550 participants.

Implications of all the available evidence

Although drug repurposing remains a feasible approach to antifungal drug discovery, sertraline should not be used as an adjunctive antifungal for cryptococcal meningitis. Until more effective antifungals are discovered, better access to the most effective existing regimens for cryptococcal meningitis is needed.

antifungal effects were observed in mice treated with sertraline and fluconazole combination therapy. In humans, sertraline readily crosses the blood–brain barrier and concentrates in the CNS,[8] and abundant clinical data support the safety and long-term use of sertraline as an antidepressant.[9]

Taken together, these studies provide evidence for the clinical potential of sertraline in the treatment of cryptococcal meningitis. This potential led to early-phase clinical trials evaluating the safety and efficacy of adjunctive sertraline on the basis of the rate of fungal clearance from cerebrospinal fluid (CSF).[7,10] In a previous open-label, dose-ranging pilot study among patients with HIV-associated cryptococcal meningitis that we did in Uganda, adjunctive sertraline at doses of 100–400 mg/day was safe, well tolerated, and appeared to improve CSF cryptococcal clearance compared with historical controls.[7] This finding was used as justification for the current study, the aims of which were to determine whether treatment with adjunctive sertraline would improve survival among adults with HIV-associated cryptococcal meningitis compared with placebo, and to assess the cost-effectiveness of adjunctive sertraline in this setting.

Methods

Study design and participants

We assessed 18-week survival among HIV-infected adults (aged ≥18 years) with first episode cryptococcal meningitis in a double-blind, randomised, placebo-controlled trial. Participants were recruited from two referral hospitals in Kampala and Mbarara, Uganda.

Diagnosis of cryptococcal meningitis was made at bedside via a positive CSF cryptococcal antigen lateral flow assay (Immy, Norman, OK, USA). Participants were included in the study if they had concurrent HIV infection and were willing to receive protocol-specified lumbar punctures. Patients were excluded if they had received three doses or more of amphotericin B, had a previous history of cryptococcal meningitis, were unable to attend scheduled outpatient visits, had jaundice or known liver cirrhosis, were receiving antidepressant medication at time of enrollment, were pregnant, or were breastfeeding.

Participants provided written informed consent at time of cryptococcal diagnosis. Approval for the clinical trial was obtained from the Uganda National Council for Science and Technology and institutional review boards in Uganda and at the University of Minnesota.

Randomisation and masking

The study enrolment process involved multiple study personnel who were involved throughout the entirety of this trial, and randomisation oversight was provided by the study coordinator. A computer-generated randomisation sequence was generated by, and accessible only to, the study statisticians and the central study pharmacist in Kampala, neither of whom were involved in enrolment or clinical care. This randomisation list was used to prepare blinded treatment boxes (inpatient) and bottles (outpatient) containing sertraline or identical placebo in tablet formulation. Patients were randomly assigned (1:1) to receive standard therapy for cryptococcal meningitis with either adjunctive sertraline or placebo, with variable block sizes of two and four and stratified according to antiretroviral therapy (ART) status (ART-experienced or ART-naive) and by enrolment site. Participants were defined as being ART-experienced if they had received

ART within 1 month before enrolment. ART status and timing has already been described in detail in a study that combined participants in this and our earlier phase 2 study.[11] All patients and clinicians providing care and assessing outcomes were masked to study treatment allocation.

Procedures

Participants received standard antifungal therapy plus adjunctive sertraline at 400 mg/day (four tablets) or placebo for 2 weeks, followed by a dose of 200 mg/day (two tablets) for the next 12 weeks before discontinuing sertraline or placebo, which were tapered over 3 weeks. Administration of study drug was by directly observed therapy via an enteral route (by mouth or crushed tablets through nasogastric tube) over the first 14 days of the study, and by self-administration thereafter. Standard antifungal therapy included protcol-mandated intravenous amphotericin B (0·7–1·0 mg/kg per day) for up to 14 days and oral fluconazole (800 mg/day, dose adjusted to 1200 mg/day if concurrently receiving rifampin) for about 4 weeks, followed by fluconazole 400 mg/day for 8 weeks of consolidation therapy and fluconazole 200 mg/day for secondary prophylaxis. The use of high-dose fluconazole as part of combination induction therapy was based on guideline recommendations in the absence of flucytosine, which is unavailable in Uganda.

Amphotericin B was generally discontinued if the baseline CSF culture was sterile after 7 days of incubation, with continuation of fluconazole and sertraline or placebo. For ART-naive participants or those on ineffective regimens, ART was initiated or switched 4–6 weeks after cryptococcal diagnosis. Participants were monitored for signs of depression throughout the study. A Center for Epidemiological Studies Depression (CES-D) score of 16 or more was interpreted as suggestive of depressive symptoms, although a diagnosis of depression required complete clinical assessment. In cases of severe depression, the protocol dictated a switch to open-label sertraline and referral to a psychiatrist for further management.

Therapeutic lumbar punctures were done routinely using manometers on day 3, day 7, and at the end of amphotericin B therapy to determine the CSF pressure and response to treatment. CSF pressure was measured using spinal manometers, and CSF was drained as necessary. Additional lumbar punctures were done as indicated for symptoms of elevated intracranial pressure and when opening pressure was 250 mm H_2O or higher during the previous days' lumbar puncture. Quantitative CSF cultures were prepared with five serial 1:10 dilutions of 100 µL of CSF, as previously described.[12,13] CSF culture sterility was defined as no growth of cryptococcus after 10 days of incubation, with a limit of detection of 10 colony-forming units (CFU) per mL.

Clinical and laboratory data were collected over an 18-week period. We used the National Institute of Allergy and Infectious Diseases Division of AIDS toxicity scale, version 2.0, to assess adverse events in all participants. Because 80% of patients were expected to have grade 3–5 adverse events with amphotericin B deoxycholate, we only reported grade 4–5 adverse events, which were expected to occur in 35–40% of patients. Most grade 4–5 adverse events were expected to be related to amphotericin B toxic effects.[7,14,15] Neurocognitive function and depression screening were assessed after 3 months, the timing of which was intended to be as close as possible to the end of the 18-week follow-up period, while still allowing scheduling flexibility given the substantial demands of neurocognitive evaluation.

The sertraline dosing regimen used in this study was based on previously published sertraline distributions in the human brain (median concentration 16·5 [IQR 13·0–21·3] times higher than in plasma),[8] the minimum inhibitory concentration (MIC) of sertraline in Ugandan isolates,[4,7] and the plasma concentrations observed in our earlier pilot study[7] to determine the probability of reaching a target concentration in brain tissue using 25 000 replicates (Microsoft Excel 2016). In a post-hoc analysis, additional sertraline plasma concentrations were measured in a sample of 106 participants randomly chosen on the basis of sample availability at 7–14 days (n=106) and at 4 weeks (n=77), as previously described.[7]

Outcomes

The primary outcome of the study was 18-week survival. Secondary outcomes were early fungicidal activity to show the cryptococcus clearance rate in CSF, cumulative incidence of grade 4–5 adverse events, 2-week CSF culture sterility, cumulative incidence of relapse or paradoxical immune-reconstitution inflammatory syndrome (IRIS), quantitative neurocognitive performance Z score (QNPZ-8) at 14 weeks,[16,17] CES-D at 14 weeks, proportion of participants diagnosed with severe depression, and event-free survival time (ie, survival without paradoxical IRIS or relapse of cryptococcal meningitis). We also planned to do a cost-effectiveness analysis of adjunctive sertraline therapy.

An independent data and safety monitoring committee oversaw trial safety. The study was designed for three interim analyses (after 25%, 50%, and 75% of participants had at least 18 weeks of potential follow-up time) and one final analysis (appendix pp8). The study statisticians were unmasked to treatment allocation and prepared the safety reports. A trial steering committee consisting of three independent members, two study investigators, and an observer provided advice on the conduct of the trial.

See Online for appendix

Statistical analysis

With a planned enrolment of 550 participants, the study was powered to detect a hazard ratio (HR) for sertraline compared with placebo of 0·65, assuming a two-sided α of 0·05 and 40% 18-week mortality in the placebo group. Under these assumptions, the power was approximately 80%, expected mortality in the sertraline group was

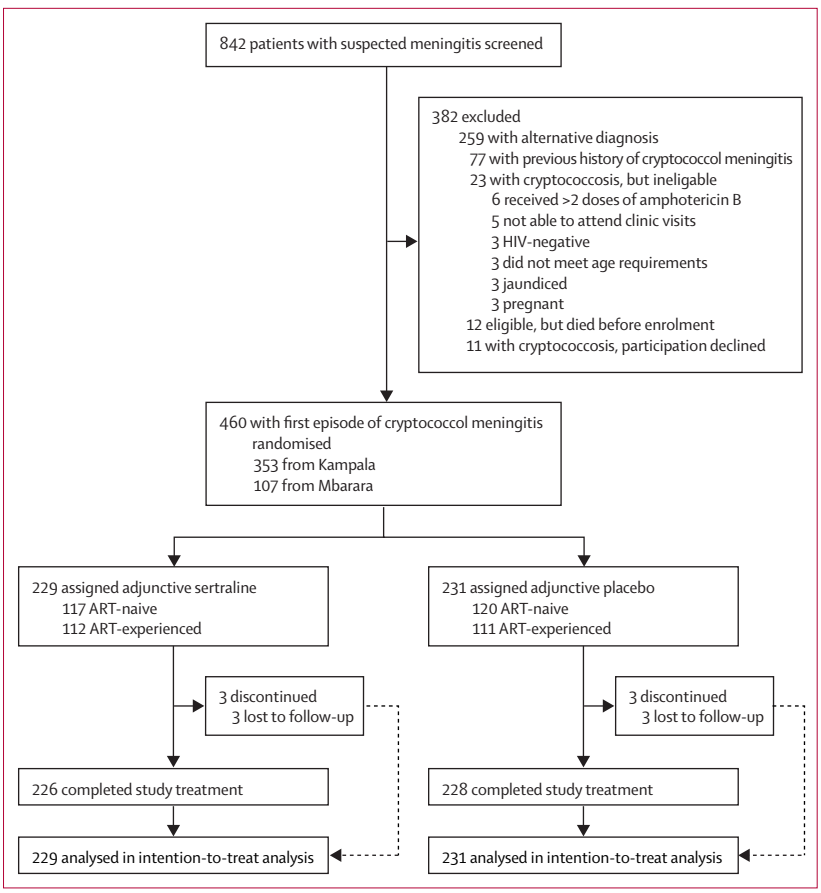

Figure 1: **Trial profile**
ART=antiretroviral therapy.

	Sertraline (n=229)	Placebo (n=231)
Demographics		
Age, years	35 (29–40)	35 (30–41)
Sex		
Female	97 (42%)	87 (38%)
Male	132 (58%)	144 (62%)
Clinical characteristics		
Weight, kg	51·5 (49·0–60·0)	54·0 (49·0–60·0)
Glasgow Coma Scale score <15	116 (51%)	105/230 (46%)
Haemoglobin, g/dL	11·3 (9·7–12·9)	11·7 (10·0–12·9)
Creatinine, mg/dL	0·7 (0·6–0·9)	0·7 (0·6–0·9)
CD4 cells per μL	17 (6–46)	13 (6–41)
Receiving antiretroviral therapy	112 (49%)	111 (48%)
Receiving tuberculosis therapy	18 (8%)	20 (9%)
Baseline CSF analysis		
CSF opening pressure, mm H_2O	248 (170–380)	270 (185–400)
CSF quantitative culture, log_{10} CFU/mL	4·7 (2·8–5·6)	4·8 (3·3–5·5)
Sterile CSF cryptococcal culture	25 (11%)	15/230 (7%)
CSF white cell count >5 cells per μL	90/222 (41%)	67/226 (30%)
CSF protein, mg/dL	47 (23–106)	42 (22–100)

Data are median (IQR) or n (%) or n/N (%). CSF=cerebrospinal fluid. CFU=colony-forming units.

Table 1: **Baseline characteristics**

28% (for a 12% absolute difference), and 188 deaths were expected.

The primary analysis was by intention-to-treat. Early fungicidal activity was assessed in participants with at least two quantitative CSF cultures in the first 18 days, 2-week CSF sterility was assessed in all patients apart from those with no growth at baseline, and paradoxical CM-IRIS was assessed in participants who were ART-naive at randomisation. All other secondary endpoints were analysed in the intention-to-treat population. Time-to-event analyses, including Kaplan-Meier curves and proportional hazards regression models, were used to summarise the pattern of mortality over 18 weeks. Preplanned subgroup analyses for mortality were done by randomisation strata (clinical site and ART use at baseline) and normal versus abnormal Glasgow Coma Scale score. A-priori subgroup analyses for mortality included sex, presence of CSF pleocytosis (white blood cell count ≤5 cells per μL *vs* >5 cells per μL), and baseline fungal burden.

The secondary outcome of early fungicidal activity was calculated for all participants with at least two quantitative CSF cultures in the first 18 days of the study with mixed effects regression models, as previously reported.[18,19] We also used a linear regression model with a random intercept for individual measurements to account for the intra-subject correlation induced by repeated measures over time. All CSF culture timepoints were used in the calculation of early fungicidal activity. Additional early fungicidal activity models were calculated, limiting the CSF cultures to three timepoints (generally days 1, 7, and 14), to compare the early fungicidal activity with that reported in other studies that limit quantitative culture timepoints, and to provide context for discussion. To determine whether fungal clearance differed by the amount of CSF removed through serial lumbar punctures, we compared early fungicidal activity by tertiles of total volume of CSF removed over the first 18 days of the study, as a post-hoc analysis.

Other secondary endpoints were summarised with cumulative incidence functions to account for the competing risk of mortality by using logistic regression and Fisher's exact methods, or were compared with general linear models or Wilcoxon rank-sum tests, as appropriate. All analyses were done with SAS version 9.3.

This study is registered with ClinicalTrials.gov, number NCT01802385.

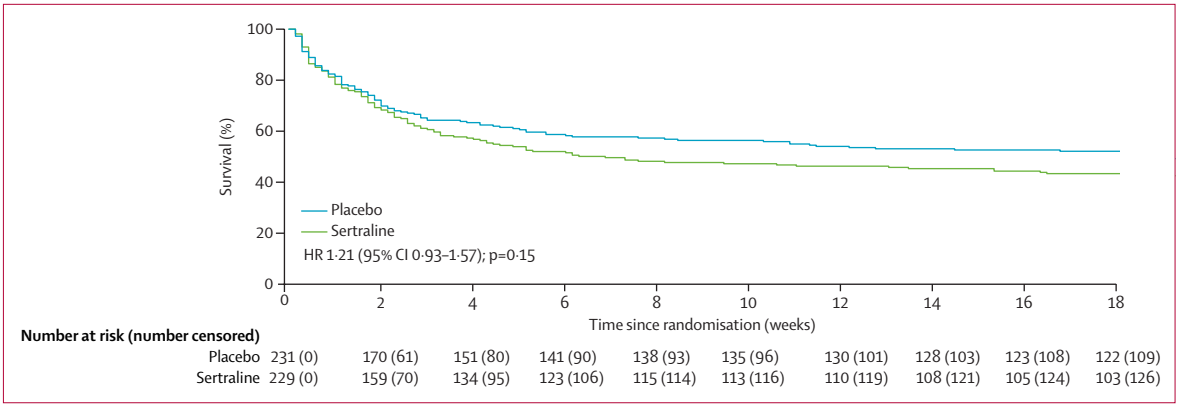

Figure 2: **Kaplan-Meier survival plot for sertraline and placebo**
HR=hazard ratio.

Role of the funding source
The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results
Between March 9, 2015, to May 29, 2017, we screened 842 patients with suspected meningitis and enrolled 460 of an intended 550 participants with cryptococcal meningitis, after which the data and safety monitoring board recommended that the trial be stopped for futility at the third interim analysis on the basis of adjunctive sertraline not showing a benefit across key outcomes. No unplanned interim analyses were done. At the time of trial suspension, 229 patients had been randomly assigned to the sertraline group and 231 to the placebo group (figure 1). Three patients in the sertraline group and three patients in the placebo group were lost to follow-up and therefore discontinued before study end. Most participants were recruited from Kampala (176 [77%] of 229 in the sertraline group and 177 [77%] of 231 in the placebo group), and slightly less than half of the participants in each treatment group were already receiving ART at time of enrolment (appendix pp 1). The proportion of participants presenting with baseline CSF pleocytosis (white blood cell count >5 cells per µL) was higher in the sertraline group than in the placebo group (table 1). All other baseline characteristics were well balanced between the two study groups. High baseline fungal burdens were common, and 9% (40/459) had sterile CSF with a positive CSF cryptococcal capsular polysaccharide antigen.

The primary outcome of 18-week mortality occurred in 120 (52%) of 229 patients in the sertraline group and 106 (46%) of 231 in the placebo group (HR 1·21 [95% CI 0·93–1·57]; p=0·15; figure 2). In the subgroup analysis, no differences in 18-week mortality were observed between treatment groups by enrolment site, ART status and timing, baseline Glasgow Coma Scale score, sex, presence of CSF pleocytosis, or initial fungal burden (appendix pp 4). Despite the imbalance in the proportion of participants presenting with baseline CSF pleocytosis, no significant interaction with treatment group in a post-hoc proportional hazards regression model was identified (p=0·76).

When using all available CSF culture timepoints collected by lumbar punctures during the first 18 days of study participation, the rate of fungal clearance from CSF was similar between groups using either general-linear or mixed-effects regression (table 2). CSF culture sterility at 2 weeks was obtained in 191 (45%) of the 420 participants with growth at baseline, and this result did not differ significantly by treatment group (table 2). Participants received a median of three (IQR 2–4, maximum 15) lumbar punctures during the first 18 days. Early fungicidal activity varied little by ART status (appendix pp 5) or by total volume of CSF removed (data not shown). When the CSF quantitative cultures used for early fungicidal activity estimations were limited to those collected by lumbar punctures on days 1, 7, and 14, the rate of fungal clearance decreased (appendix p 2).

The numbers of participants with culture-positive relapse or re-hospitalisation within 18 weeks were also similar in the two groups (table 2). The overall proportion of patients with paradoxical CM-IRIS was low, with three cases in the sertraline group and seven cases in the placebo group.

Grade 4 or grade 5 adverse events (laboratory or clinical) over the 18-week study period occurred in 31% (72 of 229 participants with 141 events) in the sertraline group, compared with 32% (75 of 231 participants with 121 events) in the placebo group (p=0·98). Most grade 4–5 adverse events were associated with amphotericin B toxicity and proportions of individual adverse events were similar between groups (table 3). Anaemia, lymphopenia, elevated creatinine, and electrolyte abnormalities (hypokalaemia, hyponatraemia) were the most common adverse events in both treatment groups. The causes of death by clinician determination was also similar between groups, with most deaths attributed to cryptococcal

	Sertraline (n=229)	Placebo (n=231)	p value*
EFA† by general linear regression, $-\log_{10}$ CFU/mL per day ‡	0·43 (0·37–0·50)	0·47 (0·40–0·54)	0·49
EFA† by mixed-effects regression, $-\log_{10}$ CFU/mL per day	0·33 (0·30–0·36)	0·33 (0·30–0·35)	0·56
2-week CSF sterility§	90/204 (44%)	101/216 (47%)	0·59
Grade 4 or 5 adverse events¶	72 (31%)	75 (32%)	0·98
Readmission to hospital‡	30 (13%)	29 (13%)	0·53
Serotonin syndrome‡	0	0	..
Culture-positive relapse	2 (1%)	2 (1%)	..
Paradoxical CM-IRIS ||	3/118 (3%)	7/122 (6%)	..
Lost to follow-up**	3 (1%)	3 (1%)	..

Data are mean (95% CI) or n (%). CFU=colony-forming units. CSF=cerebrospinal fluid. EFA=early fungicidal activity. CM-IRIS=cryptococcal meningitis-immune reconstitution inflammatory syndrome. *χ^2 test for proportions. †Among participants with at least two quantitative CSF cultures in the first 18 days (n=173 for sertraline and n=180 for placebo). ‡Not prespecified as secondary outcome in protocol. §Documented sterile CSF culture within first 18 days of study, excluding patients with no growth at baseline. ¶Combined clinical and laboratory adverse events, reported as number of patients. ||Among participants that were antiretroviral therapy-naive at randomisation. **Lost to follow-up for at least 6 weeks (no data after 12 weeks).

Table 2: **Secondary and exploratory outcomes**

	Sertraline (n=229)	Placebo (n=231)
Total grade 4–5 adverse events	141	121
Laboratory adverse events, by number of participants		
Grade 3–4, (at least 1 event)	65 (28%)	66 (29%)
Grade 4–5		
1 event	40 (17%)	48 (21%)
2 events	12 (5%)	8 (3%)
3–5 events	5 (2%)	3 (1%)
Grade 4–5, by type*		
Elevated creatinine	7 (3%)	6 (3%)
Hypokalaemia	8 (3%)	2 (1%)
Hyponatraemia	10 (4%)	11 (5%)
Hypernatraemia	2 (1%)	0
Hypomagnesaemia	0	2 (1%)
Anaemia	30 (13%)	30 (13%)
Leucopenia	1 (<1%)	0
Neutropenia	4 (2%)	0
Thrombocytopenia	1 (<1%)	0
Lymphopenia	10 (4%)	9 (4%)
Elevated ALT	0	1 (<1%)
Elevated AST	0	3 (1%)
Elevated bilirubin	9 (4%)	9 (4%)
Clinical adverse events, by number of participants†		
1 event	42 (18%)	35 (15%)
2–3 events	8 (3%)	6 (3%)

Data are n (%). ALT=alanine aminotransferase. AST=aspartate aminotransferase. *Not mutually exclusive. †Grade 4–5 clinical adverse events (clinical adverse events of grade 1–3 were not captured).

Table 3: **Adverse events**

meningitis, sepsis, or tuberculosis (appendix p 3). No cases of serotonin syndrome were observed in the trial.

We observed no difference in overall neurocognitive performance between groups at 14 weeks (mean QNPZ-8 score –1·3 [SD 0·7] for sertraline *vs* –1·4 [SD 0·9] for placebo; p=0·43). Depression scores after 14 weeks were mildly improved among patients receiving sertraline (mean CES-D score of 13·5 [SD 9·6]) compared with those receiving placebo (16·6, [10·9]; p=0·052). No participants required a switch from blinded study drug to open-label sertraline for severe depression.

A total of 124 participants experienced an event (death, relapse, or IRIS) in the sertraline group and 114 experienced an event in the placebo group, resulting in a HR for event-free survival of 1.16 (95% CI 0.90–1.50); p=0·24. As a result of the negative efficacy findings, we did not do a cost-effectiveness analysis.

Sertraline concentrations in plasma were quantified post-hoc among 106 participants between 7 days and 14 days of therapy, when a steady state was reached. The overall median sertraline plasma steady state concentration of both ART-naive and ART-experienced groups was 390 ng/mL (IQR 220–610) when receiving 400 mg/day. In participants receiving 400 mg/day without ART (n=53), sertraline concentrations were 540 ng/mL (IQR 310–680) compared with 300 ng/mL (130–500) in those receiving concurrent ART (n=53) at presentation (p<0·0001; figure 3). Sertraline concentrations were lowest for regimens that included nevirapine (median 150 ng/mL [IQR 120–190]; n=10) compared with those that included efavirenz (median 285 ng/mL [170–420]; n=26) or atazanavir or ritonavir (median 435 ng/mL [340–500]; n=6). After 2 weeks, participants received sertraline at 200 mg/day for 12 weeks. In ART-naive participants receiving 200 mg/day at 4 weeks (n=42), median sertraline concentrations were 177 ng/mL (IQR 80–335) compared with 179 ng/mL (104–344) in those receiving concurrent ART (n=35) at 4 weeks (p=0·95). Among nine participants with specimens measured between 8 and 12 weeks, the median sertraline concentration was 70 ng/mL (IQR 52–221).

Based on the MIC of sertraline on clinical isolates of cryptococcus from Uganda, observed plasma exposure, and published distribution in the brain, the proportion of patients achieving therapeutic sertraline concentrations in the brain at steady state was 92% when receiving 400 mg/day without ART, 72% with 400 mg/day and ART, and only 54% when receiving 200 mg/day with ART (appendix pp 6).

Discussion

In this phase 3 randomised trial, we found that sertraline did not reduce mortality when added to standard therapy in patients with HIV-associated cryptococcal meningitis. The consistency of the hazard ratios for survival across sites and ART strata strengthen our conclusion that

sertraline provides no survival benefit at the doses and duration tested. On the basis of the results of this trial, sertraline cannot be recommended in the treatment of cryptococcal meningitis.

We hypothesised that sertraline would improve outcomes by improving the rate of fungal clearance from the CNS. We did not observe differences in fungal clearance with the addition of sertraline to standard combination therapy with amphotericin B and fluconazole. The reasons for in-vivo sertraline inactivity are probably multifactorial and possibly associated with inadequate drug con-centrations or drug–drug interactions. On the basis of predicted sertraline distribution, the MIC of sertraline in cryptococcus, and the time required for sertraline to reach steady state, therapeutic concentrations should have been attained but might have been sustained in the brain over only a relatively short period. For patients receiving 400 mg/day, we estimate that therapeutic concentrations would have been reached only between day 7 and day 14 of treatment. This short timeframe of therapeutic exposure, during which time amphotericin B was being received, would have been unlikely to substantially improve outcomes.

Our findings are consistent with a Mexican study of 12 individuals with HIV-associated cryptococcal meningitis.[10] This study, which used the same antifungal standard therapy (amphotericin B plus high-dose fluconazole) for induction, did not show a difference in the rate of fungal clearance with adjunctive sertraline at 200 mg daily when compared with placebo given over 14 days. Although the higher doses and longer duration of sertraline administered in our trial might be expected to have improved fungal clearance, this outcome did not occur in our study in the presence of amphotericin B.

One possible reason for the failure to reach therapeutic concentrations was an under-appreciation of the interaction between sertraline and ART. Although we were unable to reach any conclusions about interactions between individual ART regimens, we observed substantially lower sertraline concentrations in the presence of ART at higher sertraline doses. We also observed lower than expected plasma concentrations of sertraline in samples collected at later timepoints (8–12 weeks). Possible explanations for low plasma concentrations at later timepoints include poor sertraline adherence in the absence of directly observed therapy or increased induction of metabolism by ART. Differences observed in plasma sertraline concentrations by ART status did not translate into differences in fungal clearance, suggesting that sertraline had little effect on fungal clearance even in ART-naive individuals.

Although we did not show a survival benefit for sertraline, we observed good safety and tolerability of sertraline despite the high doses used during the induction phase. In addition, sertraline appears to have been beneficial for treating depression, with a CES-D score 3·4 points lower in the sertraline group at week 14 than the placebo group. Consistent with other HIV trials in sub-Saharan Africa,[20,21] we found depression to be prevalent in our population. This study further emphasises the value of early depression screening and treatment when indicated among patients with HIV presenting with opportunistic infections.

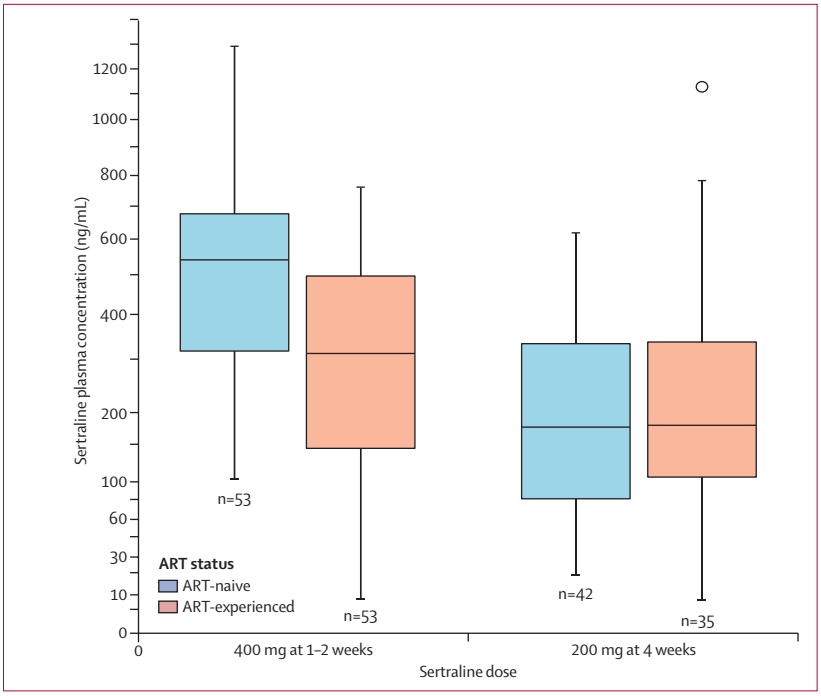

Figure 3: **Plasma concentrations of sertraline**
Steady state plasma concentrations for patients receiving 400 mg/day sertraline were measured between 7 days and 14 days of therapy, and the median was calculated. The central line of the boxes shows the median, the top and bottom of the boxes show the IQR, and the whiskers show the range (maximum to minimum). The circle indicates a single outlier. ART=antiretroviral therapy.

Because of toxicity and difficulties with the availability and administration of amphotericin B, there is a crucial need for new, well tolerated, low-cost, orally administered antifungals. Flucytosine is still not available in low-income and middle-income countries, because of costs and licensing restrictions. Agents with lower standalone fungicidal activity than amphotericin B, although not intended to be a substitute, might still be suitable for adjunctive use in combination induction therapy or for use outside the induction period, provided they can be administered orally for extended durations with low toxicity. The potential of sertraline, similar to fluconazole, is as an adjunct to more active induction therapy. However, evaluating the efficacy of candidate antifungal agents for this purpose is difficult in the presence of more potent amphotericin-B-based combination therapy. A so-called efficacy ceiling could be provided by amphotericin B that masks any potential activity of lower potency antifungals such as sertraline. Fluconazole has an important role in the treatment of cryptococcal meningitis (eg, in consolidation or maintenance therapy)

despite the lack of a proven mortality benefit, as part of combination induction therapy.[22] Following this line of reasoning, it might be more appropriate to evaluate lower potency antifungals in trials designed to measure outcomes outside the induction period, whereas trials evaluating outcomes in the induction period could be reserved for drugs with efficacy similar to amphotericin B.

A perceived improvement in fungal clearance compared with historical controls provided partial justification to test sertraline after our phase 2 trial.[7] The early fungicidal activity observed in both the sertraline and placebo groups in the current study was similar to that in our earlier dose-ranging pilot study and improved compared with historical controls receiving the same standard antifungal therapy at the same sites from 2010 to 2012 (appendix pp 7).[14] The apparent improved fungal clearance compared with historical controls seems to have resulted from a statistical anomaly due to changes in the quantity and timing of CSF culture data collected. Although the study population and standard antifungal regimen remained similar over time, the number of lumbar punctures administered per patient increased, because of the survival benefits of therapeutic lumbar punctures.[23] Specifically, in both the present study and in our previous phase 2 study[7] we did more systematic lumbar punctures, particularly on days 3 and 10, in addition to days 1, 7, and 14. Although the total volume of CSF removed from each patient over the 14-day induction period did not directly affect early fungicidal activity, an increased number of datapoints resulted in more accurate early fungicidal activity estimations and contributed to a false perception of improved fungal clearance in the phase 2 pilot study over historical controls,[7,14] when in fact there was no change. When limiting early fungicidal activity calculations to only using day 1, 7, and 14 culture data, the present trial's early fungicidal activity was $0 \cdot 34$ $\log_{10}$ CFU/mL per day, which is similar to the early fungicidal activity calculated in historical controls used as a comparison.[14]

The trial was stopped early because adjunctive sertraline did not show any clinical benefit. As a result, the treatment groups did not reach the planned size. Although the smaller sample size decreases the power for subgroup analysis, we believe the main findings of our trial are valid and generalisable across clinical settings in resource-limited and high-income countries. Although the standard of antifungal regimen care in Uganda is suboptimal because of the absence of flucytosine, participants in our trial underwent prompt lumbar puncture with comprehensive, state-of-the-art diagnostics on CSF, and received a high level of care from experienced clinicians and nurses.[24] A strength of the trial was the extensive monitoring and control of elevated intracranial pressure with serial therapeutic lumbar punctures. This trial provides further evidence that high levels of care for cryptococcal meningitis can be delivered in resource-limited settings leading to improved survival in these settings.[15,25,26]

Our trial emphasises some of the limitations of using surrogate endpoints and retrospective comparisons in advancing clinical trials, particularly when combinations of antifungals are used. Despite the findings of this trial, we remain proponents of repurposing drugs as a means of fast-tracking promising therapeutic agents for cryptococcal meningitis. Until more effective adjuvants are identified, improving access to the most effective regimens containing amphotericin B and flucytosine must remain a global priority for the treatment of cryptococcosis.

Contributors

JR designed the study, collected the data, did the primary data analysis, and wrote the initial draft of the manuscript. KHH contributed to protocol development, did primary data analysis, and provided input on subsequent drafts of the manuscript. LT, EN, EM, EEE, RK, KAP, KS, AA, DAW, MA, and AKM collected data and provided input on subsequent drafts of the manuscript. ASB and MRN contributed to data analysis and interpretation, and provided input on subsequent drafts of the manuscript. CM designed the study and provided input on subsequent drafts of the manuscript. DBM and DRB designed the study, did data analysis, and provided input on subsequent drafts of the manuscript.

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Declaration of interests

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Data sharing

All available data can be obtained by contacting the corresponding author.

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