Adjunctive Sertraline for the Treatment of HIV-Associated Cryptococcal Meningitis

Subtitle:

Improving Diagnostics and Neurocognitive Outcomes in HIV/AIDS-related Meningitis

Principal Investigators: David B Meya, David R Boulware

Protocol Champion: Joshua Rhein

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PROTOCOL SUMMARY

Title:	Adjunctive Sertraline for the Treatment of HIV-Associated Cryptococcal Meningitis
Subtitle:	Improving Diagnostics and Neurocognitive Outcomes in HIV/AIDS-related Meningitis
Type of Study:	Multicenter, double-blind randomized placebo-controlled phase III trial
Population:	HIV-infected persons with Cryptococcal Meningitis (CM)
Study Duration and Subject Participation:	550 participants will be enrolled over approximately 36 months. All participants will be followed for 18 weeks, for a total study duration of approximately 41 months.
Stratification:	By study site and ART experience (naïve versus experienced)
Description of Agent or Intervention:	Sertraline (400mg/day) for 2 weeks, then 200mg/day for 12 weeks, then tapered over 3 weeks vs. placebo; added to standard CM therapy.
Objectives:	**Primary**: To determine whether adjunctive sertraline will lead to improved 18-week survival compared to standard therapy alone. **Secondary**: 1. To compare adjunctive sertraline therapy group with the placebo control group for: A. CSF Early Fungicidal Activity (ΔCFU/mL CSF/day) B. Safety and tolerability of adjunctive sertraline (grade 4-5 adverse reactions) C. 2-week CSF culture sterility D. Incidence of CNS immune reconstitution inflammatory syndrome (IRIS) or relapse. E. Quantitative neurocognitive performance score (QNPZ-8) and Center for Epidemiologic Studies in Depression (CES-D) scale at 14 weeks.

F. Proportion diagnosed with severe depression (and switched from blinded to open-labeled study drug).

G. Event free survival (i.e. death, CM-IRIS, CM relapse).

3. Cost effectiveness analysis of adjunctive sertraline therapy

Description of Study Design:

This is a phase III randomized trial to evaluate the effect of sertraline when added to standard therapy for CM, with the hypothesis that adjunctive sertraline will lead to faster fungal clearance from the brain parenchyma and improved survival. CM diagnosis will be made via CSF cryptococcal antigen (CRAG) at time of lumbar puncture (LP) with confirmation by CSF culture. After informed consent, subjects with CM who meet eligibility requirements will be enrolled.

Subjects will be randomized to standard induction therapy with masked placebo or sertraline at 400mg/day. We will use a permutated block randomization in a 1:1 allocation stratified by clinical site (n=275 per arm). Total anticipated enrollment: 550 subjects.

CSF from the LPs at diagnosis, day 3, day 7, day 10, and day 14 will be sent for PK studies and for quantitative cryptococcal culture. Quantitative cultures will be used to calculate early fungicidal activity (EFA) over 14-days, and we will compare EFA between study arms.

Clinical Sites

Mulago Hospital, Kampala, Uganda (and affiliated sites)
Mbarara Regional Referral Hospital, Mbarara, Uganda
Ifakara Health Institute, Ifakara, Tanzania

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1 KEY ROLES

Principal Investigators:

David B Meya MBChB MMed
Infectious Disease Institute
P.O. Box 22418
Mulago Hospital Complex
Kampala, Uganda
Phone: +256.77.254.3730
Email: david.meya@gmail.com

David R Boulware MD, MPH, CTropMed
University of Minnesota
2001 6th Street SE, MTRF 3-222
Minneapolis, MN 55455 USA
Phone: +1.612.626.9546
Email: boulw001@umn.edu

Protocol Champion:

Joshua Rhein MD
University of Minnesota
420 Delaware Street SE, MMC 250
Minneapolis, MN 55455 USA
Email: joshua.rhein@gmail.com

Investigators:

Emili Letang MD, PhD
Swiss Tropical and Public Health Institute
Ifakara Health Institute (IHI), Ifakara branch
Saint Francis Referral Hospital (SFRH)
P.O. Box 53
Ifakara, Tanzania
Email: emili.letang@cresib.cat

Diana Faini MD
Swiss Tropical and Public Health Institute
Ifakara Health Institute (IHI), Ifakara branch
P.O. Box 53
Ifakara, Tanzania
Email: dfaini@ihi.or.tz

Andrew Kambugu MBChB MMed
Infectious Disease Institute
Kampala, Uganda
Email: akambugu@idi.co.ug

Conrad Muzoora MBChB, MMed
Mbarara University of Science & Technology
P.O. Box 1410,
Mbarara, Uganda
Email: conradmuzoora@yahoo.com

Kabanda Taseera MBChB, MSc, MMed
Mbarara University of Science & Technology
Mbarara, Uganda
Email: kabtash@yahoo.com

Kirsten Nielsen, PhD
University of Minnesota
420 Delaware Street SE, MMC 196
Minneapolis, MN 55455 USA
Email: knielsen@umn.edu

Study Coordinator:

Darlisha A Williams MPH
Infectious Disease Institute
Kampala, Uganda
Email: coat.trial@gmail.com

Biostatistician:

Kathy Huppler Hullsiek PhD
Division of Biostatistics
University of Minnesota
2221 University Ave SE, Suite 200
Minneapolis, MN 55414 USA
Email: kathy-h@ccbr.umn.edu

VERSION CONTROL – SUMMARY OF REVISIONS

Document	Version Date	Summary of Changes
Original protocol (version 1.0) not implemented	24 April 2013	Initial Protocol Draft
Protocol version 2.0 Phase I implemented in Kampala	13 Aug 2013	Incorporates changes suggested by IRBs Sample size enlarged Mbarara and Ifakara sites added
Protocol version 3.0	29 Jan 2014	Doses for Phase III tentatively decided as 200mg and 400mg for initial two week induction therapy Phase I activities (completed) removed from the protocol for clarity. Primary Endpoint changed to 18-week survival CSF early fungicidal activity (EFA) changed to secondary endpoint. Involvement of subjects with previous history of CM clarified
Protocol version 4.0	22 Oct 2014	Trial design is simplified to a 2-arm study testing one dose of sertraline vs. placebo. Sample size enlarged from 160 to 225 per arm to maintain statistical power if there is potential better survival (65%) in placebo group and ~10% lost to follow up.
Protocol version 4.1	22 Dec 2014	Exclusion criterion changed excluding persons receiving any antidepressant at time of study enrollment.
Protocol version 4.2	1 Sept 2015	Sample size enlarged to 275 persons per arm to maintain power with a range of survival (55%-65%) estimates. Updated list of DSMB members. Secondary endpoint of neurocognitive testing to be performed at 14 weeks only. Removal of susceptibility testing as an endpoint. This was accomplished during the pilot study.

2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Primary Study Hypothesis

Cryptococcal meningitis (CM) has emerged as one of the most frequent and deadly opportunistic infections in HIV patients, with a global burden estimated at nearly 1 million cases annually.[1] Early mortality from HIV-associated cryptococcal meningitis remains unacceptably high, in large part due to the high cost, toxicity, and relatively limited repertoire of effective antifungals. Furthermore, the rate of fungal clearance from cerebrospinal fluid for currently available CM treatment regimens remains low, meaning that sterilization after 2 weeks of induction therapy cannot always be ensured.[2] For these reasons, the identification of additional antifungals effective for the treatment of CM is of utmost importance.

Recent evidence suggests that the commonly used selective serotonin receptor inhibitor (SSRI) sertraline provides potent *in vitro* fungicidal activity against *Cryptococcus neoformans*[3] and readily crosses the blood-brain barrier in animal studies.[4] Taken together, sertraline offers a promising therapeutic option for CM. We hypothesize that sertraline added to standard CM induction therapy will result in increased early fungicidal activity (EFA), resulting in faster rate of fungal clearance and better clinical outcomes.

2.2 Burden of Cryptococcal Meningitis

Cryptococcal meningitis has become the most common cause of adult meningitis in many parts of Africa,[5] where cryptococcosis now rivals tuberculosis in all-cause mortality.[1] While long-term survival has improved with widespread use of antiretroviral therapy in high-income countries, early mortality remains high.[6] Furthermore, expanding access to antiretroviral therapy in resource-limited settings has not yet led to compelling improvements in mortality, with 10-week mortality rates between 24% and 37%, even under optimal research conditions.[7,8] Early mortality rates are often >50% in routine practice where access to diagnostics or medications is limited or unavailable, intracranial pressure is uncontrolled, or in settings where other barriers to the management of cryptococcal meningitis exist.[9-12]

2.3 Treatment of Cryptococcal Meningitis

Standard treatment for CM is divided into three phases: a two-week induction phase, followed by an eight-week consolidation phase, and an extended maintenance phase thereafter for secondary prophylaxis.[13] Table 1 outlines the treatment recommendations for cryptococcal meningitis in HIV-infected individuals. Historically, international treatment guidelines have been informed primarily by large multisite clinical trials with endpoints of treatment success and survival at 2 weeks and 10 weeks.

Table 1: Antifungal Treatment Recommendations for Cryptococcal Meningitis in HIV–Infected Individuals

	Induction	Consolidation	Maintenance
Timeline	First 2 weeks	Next 8 weeks	Until immune reconstitution[a]
Preferred Regimen	Amphotericin B[b] (0.7–1.0 mg/kg per day) plus flucytosine (100 mg/kg per day)	Fluconazole 400–800 mg per day[c]	Fluconazole 200 mg per day[e]
Alternative Regimens[d]	Amphotericin B (0.7 mg/kg per day) for plus fluconazole (800 mg per day)		
	Fluconazole (1200 mg per day) plus flucytosine (100 mg/kg per day) for 6 weeks		
	Fluconazole (1200 mg per day) for 10-12 weeks		

Notes: [a] CD4 cell count 100 cells/mL, and low or nondetectable viral load for 3 months with minimum of 1 year of antifungal therapy

[b] Liposomal amphotericin B (4 mg/kg per day) may be substituted, listed in order of highest recommendation top to bottom

[c] Some experts recommend fluconazole doses of 800 mg per day until culture is known to be negative (see text)

[d] In clinical situations in which primary recommendations are not available

[e] Itraconazole (400 mg per day) or amphotericin B (1 mg/kg per week) if fluconazole intolerant, though inferior regimen

CM mortality can be reduced by the use of high-dose amphotericin B (0.7-1.0 mg/kg/day), alongside aggressive inpatient management of raised intracranial pressure.[14] In many settings, standard induction therapy for CM includes amphotericin B for up to 14 days combined with high-dose fluconazole (800mg-1200mg). The ability of amphotericin to rapidly and consistently sterilize the cerebrospinal fluid (CSF) of patients with CM suggests that amphotericin should be central to any induction strategy.[15] Substantial barriers exist in adhering to the accepted guidelines in resource-limited areas, where cryptococcal meningitis is most prevalent. These include costs and availability of drugs, intravenous (IV) drug administration, which requires hospitalization, co-administration of IV fluids and supplemental electrolytes, and the severe and potentially life-threatening toxicity of amphotericin, which necessitates rapid and reliable laboratory monitoring.[16,17] For these reasons, amphotericin is generally reserved for life-threatening fungal infections, and in the case of CM, limited to the induction period. Many centers in Africa consequently find it difficult to adhere to recommended guidelines for CM induction therapy. Identifying more effective and less toxic adjunctive antifungal therapy is required to allow for faster fungal clearance during the induction phase, which could lead to less dependence on completing a full 14-day course of amphotericin.

While the combination of amphotericin and flucytosine appears to be rapidly fungicidal and may have a lower risk of treatment failure than alternative 14-day regimens,[18,19] the added benefit of flucytosine as a second agent over other antifungals continues to be debated.[20,21] Flucytosine is also associated with severe side effects including hepatotoxicity, bone-marrow depression, and renal toxicity when co-administered with other nephrotoxic drugs such as amphotericin.[22] Furthermore, flucytosine is prohibitively expensive and generally unavailable in resource-limited settings, where CM is most prevalent. Fluconazole, in contrast to amphotericin, is generally well-tolerated and readily penetrates the central nervous system (CNS).[23] Consolidation and subsequent maintenance therapy therefore relies on extended courses of oral fluconazole (Table 1). At typical doses (≤400 mg/day), however, fluconazole is fungistatic rather than fungicidal, confirmed in a well-designed clinical trial demonstrating no change in fungal growth by serial quantitative cultures over 2 weeks with 400 mg/day of fluconazole.[15] Even when used in higher, more fungicidal doses (800-1200 mg/day)[10] as currently recommended for the treatment of CM, fluconazole adds a marginal contribution to overall fungal clearance when used together with amphotericin. Fluconazole monotherapy, which is still practiced in some areas of the

world where amphotericin use is limited or impossible to administer, leads to suboptimal clearance, resistance and symptomatic relapse.[2,24] Stronger and less toxic oral antifungal regimens for the treatment of CM are urgently needed.

2.4 Recognition and Development of New Antifungals

Given the increasing worldwide importance of invasive fungal infections, including though not limited to *Cryptococcus neoformans*, there has been a large push to expand the current armamentarium of antifungals used to treat fungal infection. The ideal antifungal agent for systemic infections would:

1. Be fungicidal, rather than fungistatic
2. Have a novel mechanism of action
3. Be well tolerated
4. Have few drug interactions
5. Have good penetration into infected body compartments
6. Be affordable

Recognizing the critical need for new antifungals against invasive fungal infections, extensive work is underway to validate new targets and develop new antifungals. The echinocandin class of antifungals, for example, is one noteworthy result of these increased development efforts. Unfortunately, the echinocandins are not active against *C. neoformans*, and few antifungals are currently in the development pipeline. Given the inherent challenges involved in *de novo* drug development, other groups have turned to existing clinical compounds in an attempt to identify known drugs with antifungal properties.[25,26] Insomuch as existing pharmaceutical and safety data already exists, investigation into their clinical use as antifungals is greatly accelerated, offering a particular benefit in the development of new therapies for globally neglected fungal diseases such as cryptococcal meningitis. This approach offers a shortcut from bench to bedside, especially when it involves a new indication for an existing and widely-used marketed drug, such as sertraline.

2.5 Antifungal Properties of Sertraline

The first indication that sertraline exhibits antifungal properties came during observations from a clinical setting in which patients were observed to have resolution of recurrent vulvovaginal candidiasis during periods in which they were concurrently being treated with sertraline for premenstrual dysphoric disorder.[27] Subsequent in vitro studies demonstrated that sertraline was fungicidal against both *Candida* and *Aspergillus* species,[27,28] though at MICs higher than what would likely be achievable in serum using standard human doses of sertraline. More recently, Zhai *et al*, by using an approach of broad screening of known clinical compounds, have confirmed the modest inhibitory effects of sertraline against *Aspergillus nidulans in vitro*.[3] When *in vitro* susceptibility testing was extended to pathogenic yeasts, particularly potent antifungal activity was observed against *C. neoformans*. The recognition of sertraline as an antifungal subsequently led to additional studies, including further *in vitro* studies using combination therapy with fluconazole, *in vivo* studies using a murine model, and preliminary studies to investigate the antifungal mechanism of sertraline.[3] The methods, specific findings, and conclusions of these studies are summarized in Table 2.

Table 2: Major Findings Regarding Anti-Cryptococcal Properties of Sertraline

Major Conclusions	Methods	Specific Findings
1. Sertraline is fungicidal against *C. neoformans in vitro*	• Susceptibility testing of 24 distinct cryptococcal strains • Time course assay comparing fungal growth in nutrient- rich vs nutrient-depleted media	• MIC_{90} ranged between 2-6 µg/mL • MFC ranged between 6-10 µg/mL • Fungal killing was independent of cell proliferation
2. Fungicidal activity of sertraline is additive when combined with fluconazole	• Comparison of sertraline and fluconazole susceptibilities • Time course assay comparing monotherapy vs combo therapy • Quantification of additive effects by calculating FFCI	• Combination therapy accelerated clearance • Larger and clearer halo in disks containing combo therapy • Effects of combo therapy were synergistic (FFCI < 0.5) or additive (FFCI 0.5 - 1.0) in all 24 strains tested
3. Sertraline is effective against *C. neoformans in vivo* in a murine model	• Mice were assigned to 1 of 4 treatment arms† and intravenously infected • Quantitative cultures obtained from suspensions of homogenized tissue 4 days after being infected	• Sertraline reduced fungal burden in brain with efficacy similar to fluconazole • The most potent anti-fungal effects were observed with combination therapy
4. Sertraline interferes with translation in fungal cells	• Whole-genome deletion screening (using collection of mutant *Saccharomyces cerevisiae*) for sertraline-sensitive or sertraline-resistant mutants • Gene ontology to identify processes affected by sertraline	• Genes related to protein synthesis were highly enriched in sertraline-resistant strains • Mutant that was most sensitive to sertraline contained disrupted gene for the translation initiation factor Tif3
5. Protein synthesis is inhibited in a *C. neoformans* cell-free system	• *In vitro* translation assays utilizing *C. neoformans* cell extract as translation machinery for luciferase mRNA • Sertraline added to mixture in dose-dependent manner	• Sertraline inhibited translation efficiency in a dose- dependent manner • Translation activity 50% when at 30.6 µg/mL • No translation when at 122.4 µg/mL

MIC = minimum inhibitory concentration; MFC = minimum fungicidal concentration; FFCI = fractional fungicidal concentration index
†Experimental arms of in vivo murine model: 1. Control group (no drug); 2. Sertraline (15 mg/kg/day, started 7 days *prior to* infection) monotherapy; 3. Fluconazole (15 mg/kg/day, started 24 hours *after* infection) monotherapy; 4. Combination of sertraline (#2) and fluconazole (#3) above.

In the study summarized above (Table 2), sertraline inhibited *C. neoformans* with an MIC between 2-6 µg/mL. In addition, sertraline appeared to be fungicidal since killing was independent of cell proliferation (Figure 1A). The combination of sertraline and fluconazole led to lower MICs and accelerated fungal clearance at a greater rate than either drug alone. Quantifying these effects using the fractional fungicidal concentration index (FFCI), the combination of sertraline and fluconazole was either additive or synergistic in all strains tested. When pretreated for 7 days at a dose of 15 mg/kg/day, sertraline was also effective against *C. neoformans* in an *in vivo* model of experimentally infected mice. The inhibitory effect of sertraline was particularly potent in the brain of infected mice, with efficacy similar to fluconazole (Figure 1B). The most potent anti-fungal effects were observed in mice treated with sertraline + fluconazole combination therapy.

Additional studies to ascertain the antifungal mechanism of sertraline suggest that translation may be inhibited in *C. neoformans*. In whole-genome deletion screening of *Saccharomyces cerevisiae* for instance, genes related to protein synthesis were highly enriched in sertraline-resistant strains (Figure 1C). Mice containing a disrupted gene for the translation initiation factor Tif3, on the other hand, were highly sensitive to sertraline. Protein synthesis was also inhibited in a *C. neoformans* cell-free system, where sertraline inhibited translation efficiency in a dose-dependent manner.

Figure 1: Antifungal Properties of Sertraline

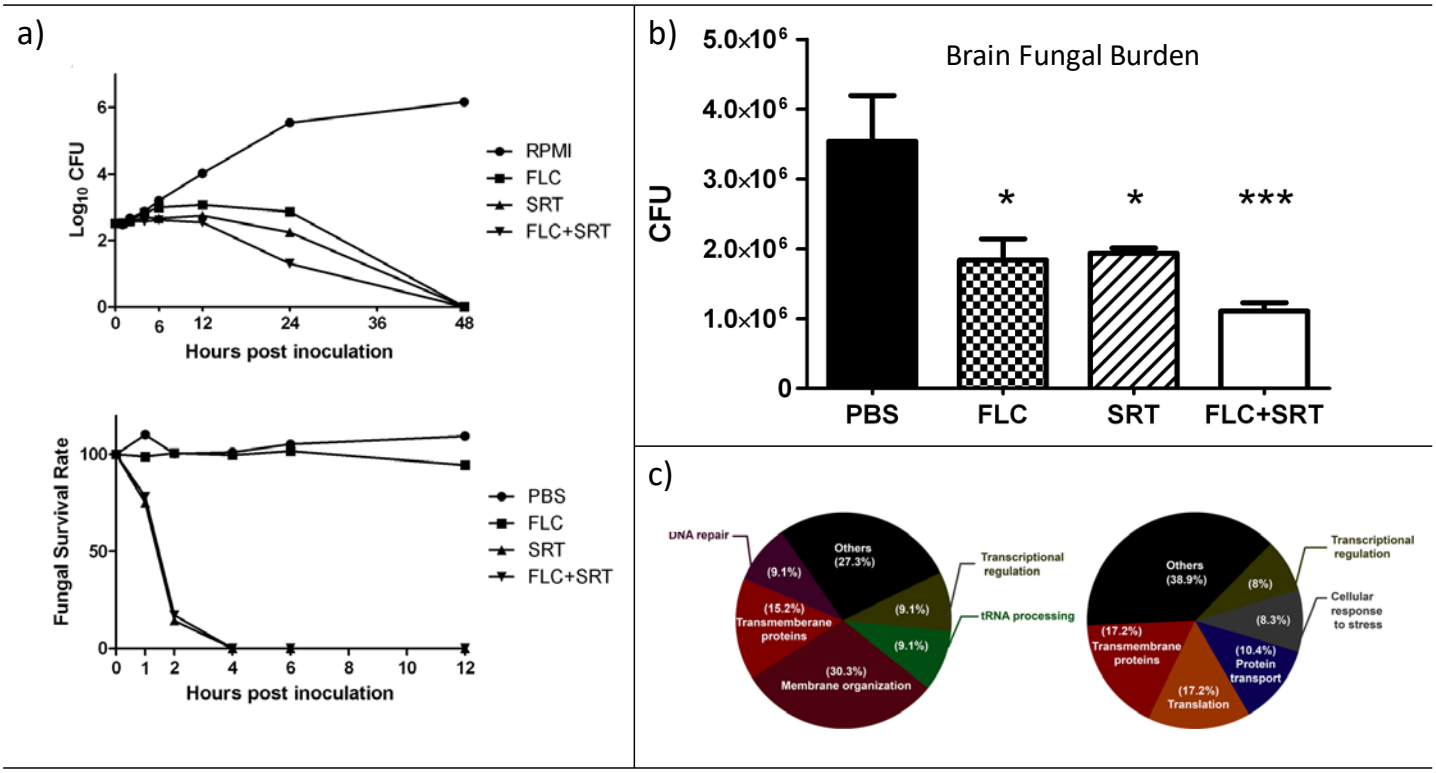


Figure 1. Antifungal properties of sertraline. **(a)** Sertraline is fungicidal against both proliferative and quiescent Cryptococcus. Cells were inoculated into media and cultured without any drug (control), or in the presence of fluconazole (FLC, 8 µg/ml), sertraline (SRT, 10µg/ml), or a combination of these two drugs. The upper chart shows proliferating cells inoculated into agar, while the lower chart shows inactive cells inoculated into PBS buffer. **(b)** Sertraline reduces the fungal burden alone or in combination with fluconazole in vivo. Brains of mice from different treatment groups were dissected and homogenized. The suspensions were diluted serially and the fungal burden was determined by calculating CFU. Sertraline alone or in combination with fluconazole significantly reduced the fungal burden. **(c)** Gene ontology analysis of the S. cerevisiae genes involved in sertraline tolerance (left pie chart) or susceptibility (right pie chart).

2.5.1 Pharmacokinetics of Sertraline

Sertraline hydrochloride is an antidepressant of the selective serotonin reuptake inhibitor (SSRI) class. Current indications for sertraline include major depression, obsessive-compulsive disorder, panic disorder, and social anxiety disorder. SSRIs are currently among the most prescribed drug classes worldwide, and sertraline in particular remains one of the most prescribed antidepressants on the U.S. retail market, with nearly 30 million prescriptions in 2007.[29]

While the efficacy of sertraline for depression is similar to that of older antidepressants, the favorable side effect profile of the SSRI class compared to tricyclic antidepressants has led to rapid and widespread adoption as first line therapy. Differences between SSRIs antidepressants are subtle, and if present at all are mostly confined to relatively mild side effects.[30]

Sertraline is slowly absorbed following oral administration and undergoes extensive first-pass oxidation to form a weakly active metabolite, which can accumulate to a greater concentration in plasma than the parent drug at steady state. The main site of metabolism is the liver, and drug clearance of sertraline can be markedly

reduced in patients suffering from liver impairment (e.g., cirrhosis). In contrast, the pharmacokinetics of sertraline do not differ significantly between healthy controls and patients suffering from renal impairment. Since it is largely excreted via the kidneys, with a half-life ranging from 22–36 hours, once-daily administration is therapeutically effective. Steady-state plasma concentrations vary widely, up to 15-fold, in patients receiving usual antidepressant dosages between 50 and 150 mg/day.[31,32] No data exists to support useful correlations in plasma concentration on therapeutic or adverse effects.

Sertraline has minimal inhibitory effects on the major cytochrome P450 enzymes, and few drug-drug interactions of clinical significance have been documented (Section 6.1).[33] Abundant clinical data supports the safety of long-term use of this as an antidepressant at doses between 50-200mg.[34-36] Like other selective serotonin reuptake inhibitors, sertraline is relatively safe in over-dosage.[37,38]

Of particular interest in regards to its use as an antifungal for the treatment of CM, previous pharmacokinetic (PK) studies of sertraline in animals suggest that sertraline concentrations in the CNS are 20- to 50- fold higher than its serum concentration.[4] While drug levels that can be reached in plasma using standard human doses are unlikely to reach cryptococcal MIC for sertraline observed *in vitro*, concentrated levels in the CNS could meet or exceed the MICs for sertraline observed in previous *in vitro* studies.[3] For example, achievable serum concentrations of sertraline using standard therapeutic regimens for psychiatric indications (50-200 mg/day) are 55-250 ng/ml.[39] Therefore, at higher therapeutic doses, CNS concentrations would be expected to reach the 2-6 μg/mL MIC for sertraline observed in the Zhai *et al* study. **Table 3** summarizes previous sertraline PK studies, and reflects observed serum concentration at varying doses of oral sertraline.[40-42]

Table 3: Sertraline Pharmacokinetic Studies

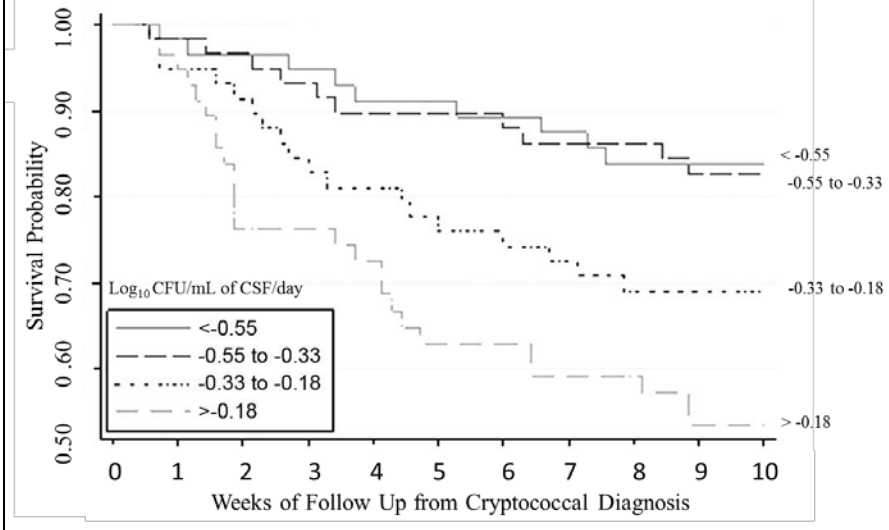
					Serum	Predicted CNS Concentration	
Study	**Notes**	Dose (mg/day)	n	Timing	μg/L	20X - 50X μg/L	20X - 50X μg/mL
Saletu *et al.*	Mean peak concentration	100	10	Single dose	54.5	1090 - 2725	**1.1 - 2.7**
		200	10		105.4	2108 - 5270	**2.1 - 5.3**
		400	10		253.2	5064 - 12660	**5.1 - 12.7**
Ronfeld *et al.*	Young male →	200	11	After 21 days	118.0	2360 - 5900	**2.4 - 5.9**
	Young female →	200	11		166.0	3320 - 8300	**3.3 - 8.3**
	Elderly male →	200	11		135.0	2700 - 6750	**2.7 - 6.8**
	Elderly female →	200	11		147.0	2940 - 7350	**2.9 - 7.4**
Lundmark *et al.*	Mean steady state trough	50	156	Clinical samples of patients taking sertraline	40.0	800 - 2000	**0.8 - 2.0**
		100	90		66.0	1320 - 3300	**1.3 - 3.3**
		150	23		127.0	2540 - 6350	**2.5 - 6.4**
		200	15		93.0	1860 - 4650	**1.9 - 4.7**

Obtaining brain or CSF samples from human subjects for pharmacokinetic studies in impractical in most research settings. Attainable concentrations of sertraline in the CNS of human subjects have therefore not been directly studied previously and as a result predicted CNS concentration must be inferred from animal models. The treatment of CM, however, which requires serial lumbar punctures to control elevated intracranial pressure, provides an ideal setting for studying drug concentrations in CSF. An additional benefit of performing PK studies on CSF from patients with CM is the ability to detect drug concentrations in a targeted population that might be expected to have different pharmacokinetic profiles compared to healthy volunteers.

2.6 Early Fungicidal Activity

Rate of fungal clearance, or early fungicidal activity (EFA), provides a measure for evaluating the efficacy of induction therapy for CM. Bicanic et al have demonstrated in a pooled series of Phase II clinical trials that the rate of cerebrospinal fluid sterilization correlates with clinical outcomes (**Figure 2**). Specifically, there is an association between early fungicidal activity during induction therapy and the 2-week and 10-week survival after cryptococcal meningitis.[43]

Figure 2: Microbiologic Rate of CSF Cryptococcal clearance association with Survival



Kaplan-Meier survival curve by rate of clearance of infection, categorized by quartiles. More rapid clearance, measured by early fungicidal activity (EFA; measured in Log_{10} CFU/mL of CSF/day), leads to improved early survival. Patients in top two quartiles for rate of clearance (EFA > 0.33 Log_{10} CFU/mL/day) had a higher survival probability than patients in lower quartiles. (Modified from: Bicanic T, Muzoora C, Brouwer AE, et al. *Clin Infect Dis* **2009**; 49: 702-09.)

Quantitative cryptococcal culture clearance from cerebrospinal fluid is generally logarithmic over the first 2 weeks, thus the clearance can be estimated by linear regression of log_{10} colony-forming units (CFU) per mL of cerebrospinal fluid per day of therapy as a unit of measure (i.e. change in log_{10} CFU/mL cerebrospinal fluid/day). This new approach has allowed for small innovative clinical trials to estimate the early fungal activity of newer induction regimens with dramatically smaller sample sizes, using early fungal activity as a surrogate marker. **Table 4** summarizes early fungal activity data from several recent trials comparing the early fungicidal activity of different induction regimens.

Table 4 Trials comparing early fungicidal activity of induction treatment regimens for cryptococcal meningitis

Induction Regimen	EFA	± SD	n
AmB + 5FC	-0.41	0.22	21
AmB + fluc (800 mg daily)	-0.38	0.18	22
AmB + fluc (1200 mg daily)	-0.41	0.35	23
AmB + voriconazole	-0.44	0.20	13
AmB (5 days) + fluc (1200mg daily)	-0.30	0.11	30
AmB + 5FC	-0.49	NA	30
AmB + 5FC + INF-γ	-0.64	NA	60
5FC + fluconazole (1200 mg daily)	-0.28	0.17	21
Fluconazole (1200 mg daily)	-0.11	0.09	20
AmB (7 days) + fluc (1200 mg daily)	-0.39	0.20	19
AmB (7 days) + fluc (1200 mg daily) + 5FC	-0.49	0.15	18
AmB (0.7 mg/kg/day) + 5FC	-0.45	0.16	28
AmB (1 mg/kg/day) + 5FC	-0.56	0.24	29
Fluconazole (1200 mg daily)	-0.18	0.11	30
Fluconazole (800 mg daily)	-0.07	0.17	30
AmB (1 mg/kg/day)	-0.48	0.28	49
Fluconazole (400 mg daily)	-0.02	0.05	5
AmB (0.7 mg/kg/day)	-0.31	0.18	14
AmB (0.7 mg/kg/day) + 5FC	-0.54	0.19	12
AmB + fluconazole (400 mg daily)	-0.39	0.15	11
AmB + 5FC + fluc (400 mg daily)	-0.38	0.13	15

Abbreviations: EFA, early fungicidal activity (log CFU/mL CSF/day); AmB, amphotericin B (0.7 or 1 mg/kg/day or as indicated); 5FC, flucytosine (25 mg/kg 4 times daily); Fluc, fluconazole (doses indicated); Voriconazole (300 mg twice daily; 400 mg twice on day 1); INF-γ , interferon-gamma (100 µg subcutaneously, 2 or 6 doses over induction period).

2.7 Rationale for Sertraline Adjunctive Therapy

In addition to providing a basis for comparing the efficacy of different induction regimens, EFA can be used to estimate the time required to sterilize the cerebrospinal fluid in CM. As **Figure 3** demonstrates, the probability of sterilizing the CSF by day 14 of induction therapy is dependent both on the initial fungal burden and the early fungal activity of the induction regimen used. This is further influenced by the host response to infection, with a paucity of CSF inflammation (e.g. normal CSF white blood cell count, normal CSF protein level, and lower IFN-γ levels in cerebrospinal fluid) being associated with worse microbiologic clearance.[9,43-45]

Figure 3: Predicted CSF Mean Clearance for various Cryptococcal Induction Treatment Regimens

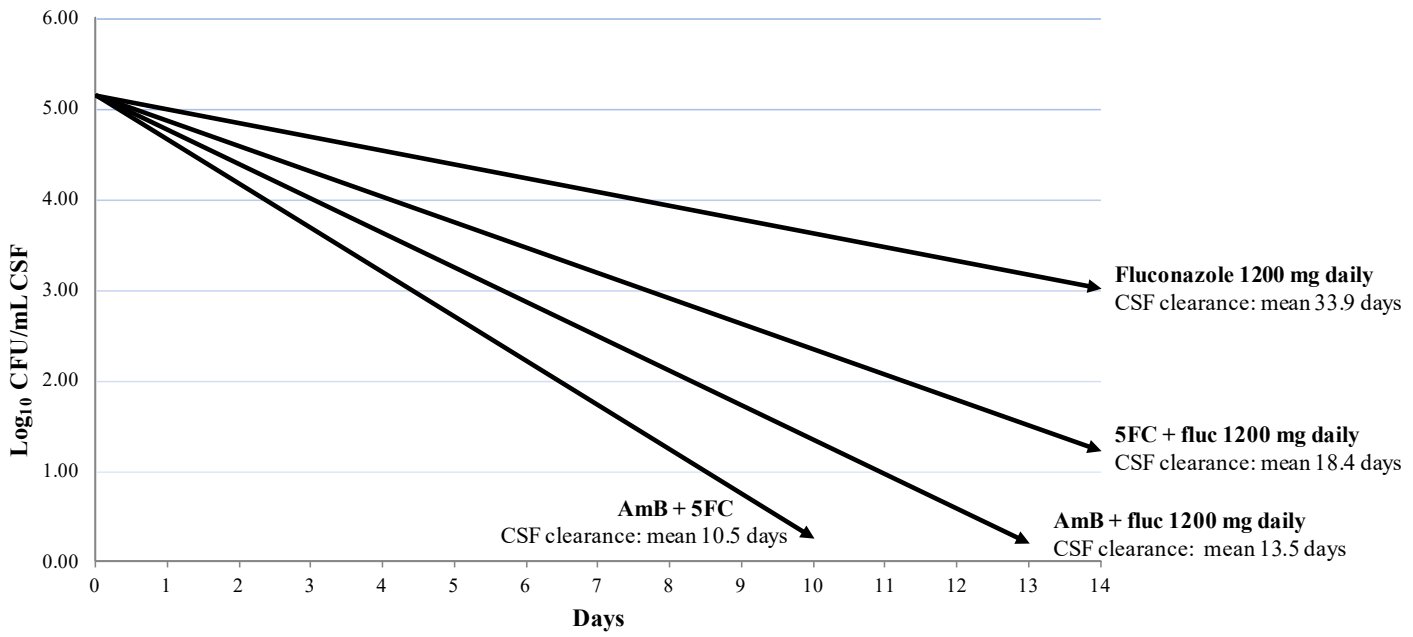


Figure 2 demonstrates the predicted mean clearance of Cryptococcus from CSF as a function of time for various induction regimens used for CM, based on early fungicidal activity (refer to Table 3). The initial cerebrospinal fluid colony count in this figure (140,000 CFU/mL = 5.15 log CFU/mL) is the median value from the recent COAT trial (Cryptococcal Optimal ART Timing Trial, NCT01075152). As demonstrated, the average time required for cerebrospinal fluid sterilization can be predicted based on the initial quantitative culture of cerebrospinal fluid and the induction regimen selected. This concept provides the basis for either prolonged induction therapy or higher consolidation therapy (e.g. fluconazole 800–1200 mg/day).

Abbreviations: 5FC, flucytosine; AmB, amphotericin B; fluc, fluconazole.

More effective regimens with higher EFAs are expected to clear cryptococcal infection more rapidly. As figure 3 suggests, the combination of amphotericin and flucytosine is therefore the most effective regimen currently being used. Given the unavailability of flucytosine in resource-limited settings, fluconazole is more often used as part of initial combination therapy with amphotericin. Maximizing the efficacy of this regimen is therefore of central importance insofar as a greater percentage of patients would be expected to clear their infection during induction therapy. Considering the toxicity of amphotericin and the inherent difficulties encountered during its administration, a faster rate of clearance could also lead to shortened courses of amphotericin, minimizing its toxic effects as well as costs and nursing commitments associated with lengthy amphotericin-based regimens.

The additive effects observed when sertraline was combined with fluconazole in vitro and in animal models suggests that this may be an ideal candidate in maximizing the rate of fungal clearance. Given its fungicidal

properties, safety profile, lack of drug interactions, likely novel mechanism of action, and excellent penetration and concentration in the CNS, sertraline meets many of the characteristics outlined above for an ideal adjunct antifungal against *C. neoformans*. However, sertraline has not yet been tested in a clinical trial to determine if the in vitro, animal, and mechanistic studies outlined above can translate to real-world benefit in humans. For these reasons, further clinical trials must be performed. Sertraline, a generic, off-patent oral medicine could have the potential to revolutionize cryptococcal care in Africa where an estimated ½ million patients die every year. If effective against cryptococcosis, sertraline has the potential to decrease the hundreds of thousands of deaths per year due to CM.

2.8 Potential Risks and Benefits

2.8.1 Potential Risks

Subjects will be receiving standard induction therapy for CM, with the exception that sertraline will be added in the experimental arms. Abundant clinical data supports the safety of long-term use of sertraline as an antidepressant at doses between 50-200mg,[34-36] few drug-drug interactions of clinical significance exist, and it is relatively safe in over-dosage.[37,38] Despite this, sertraline has not previously been used in humans specifically as an antifungal for the treatment of CM. Since its benefit as an antifungal cannot be fully determined until a trial has been undertaken, there is a risk that subjects could receive a drug without clear indication. Although sertraline is a relatively safe drug overall, adverse reactions can still occur, as with any drug. Refer to section 6.1 for a discussion on the known drug interactions and adverse effects associated with sertraline.

Recommended dosages of sertraline for psychiatric use in humans range from 50-200 mg/daily. We will be using a dose of 400 mg/day during induction therapy in our experimental arm, which is higher than the recommended dose for the treatment of psychiatric conditions in humans. However, this maximum dose recommendation is based primarily on a lack of additional therapeutic benefit balanced against an increased incidence of relatively common though generally mild side effects, rather than a true concern over dangerous toxicity. For example, in a series of 52 patients evaluated for sertraline-only overdose (mean ($\pm$SD) dose 727 $\pm$686 mg), symptoms were generally mild CNS, cardiovascular, or gastrointestinal effects.[46] There was no serious toxicity, and 34 cases in this series had no symptoms at all. In the higher dose arm, we would therefore expect an increased incidence of side effects. However, we believe that this increased occurrence of mild side effects would be offset by a more potent antifungal regimen for CM, an often deadly disease.

A gradual titration upwards is generally recommended in clinical practice to reduce the occurrence of adverse events that may be encountered at higher doses of sertraline. This study design, however, bypasses any upward titration in an attempt to reach therapeutic levels in the CNS more rapidly. As a result, it is expected that more side effects will be experienced in this trial than what might typically be seen in clinical practice. Nevertheless, for the reasons discussed above, we believe that the potential benefits of better treatment for a deadly fungal infection outweigh the risk for increased adverse events from sertraline.

2.8.2 Known Potential Benefits

The most important benefit for participating in this study is the potential to receive a more effective induction regimen for cryptococcal meningitis, thereby reducing early mortality. It is hoped that more

effective treatment for CM in the future will lead to better clearance of infection, less likelihood of developing resistance or disease relapse, and shorter, less toxic induction regimens. The addition of sertraline to standard CM therapy may also decrease the incidence of relapse disease and IRIS, an important complication of ART therapy in patients with CM.

Another potential benefit of enrolling into this trial is better recognition and treatment of underlying depression. Mental disorders have been identified as one of the leading causes of health disability in sub-Saharan Africa and worldwide.[42,47] In Uganda, for example, major depressive disorder in HIV-positive persons is exceedingly common, though often not diagnosed and poorly treated.[48] This problem is often exacerbated by limited availability and affordability of access to medicines, including SSRIs. Patients enrolled in this trial will be assessed for depression and sertraline will be provided. While the trial is intended to evaluate the antifungal properties of sertraline, it is also a first line therapy for depression and subjects that are found to have depressive disorder will be encouraged to continue this medication at conclusion of the trial if receiving benefit from a mental health standpoint.

3 OBJECTIVES

3.1 Study Objectives

3.1.1 Primary Objective

To determine whether adjunctive sertraline will result in improved 18-week survival compared to standard therapy for CM alone.

3.1.2 Secondary Objectives

To compare the adjunctive sertraline vs. placebo group for fungal clearance, pharmacokinetics, microbiologic susceptibilities, paradoxical CM-IRIS, culture-positive relapse, safety, neurocognitive and depression outcomes, and potential cost-benefit.

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measures

1. 18-week survival.

3.2.2 Secondary Outcome Measures

1. To compare adjunctive sertraline therapy with the placebo control group for:
    A. CSF early fungicidal activity (EFA) during induction treatment: as measured by the change in $\log_{10}$ CFU/mL of CSF/day;
    B. Quantitative neurocognitive performance score (QNPZ-8) and Center for Epidemiologic Studies in Depression (CES-D) scale at 14 weeks;
    C. 2-week CSF culture sterility;
    D. Cumulative incidence of CNS cryptococcal-related IRIS or relapse
    E. Adverse reactions (grade 4-5);

F. Proportion diagnosed with severe depression (and switched from blinded to open-labeled study drug);

G. Event free survival time for all-cause mortality, paradoxical CM-IRIS, and/or CM-relapse.

2. Conduct a cost effectiveness analysis of adjunctive sertraline therapy.

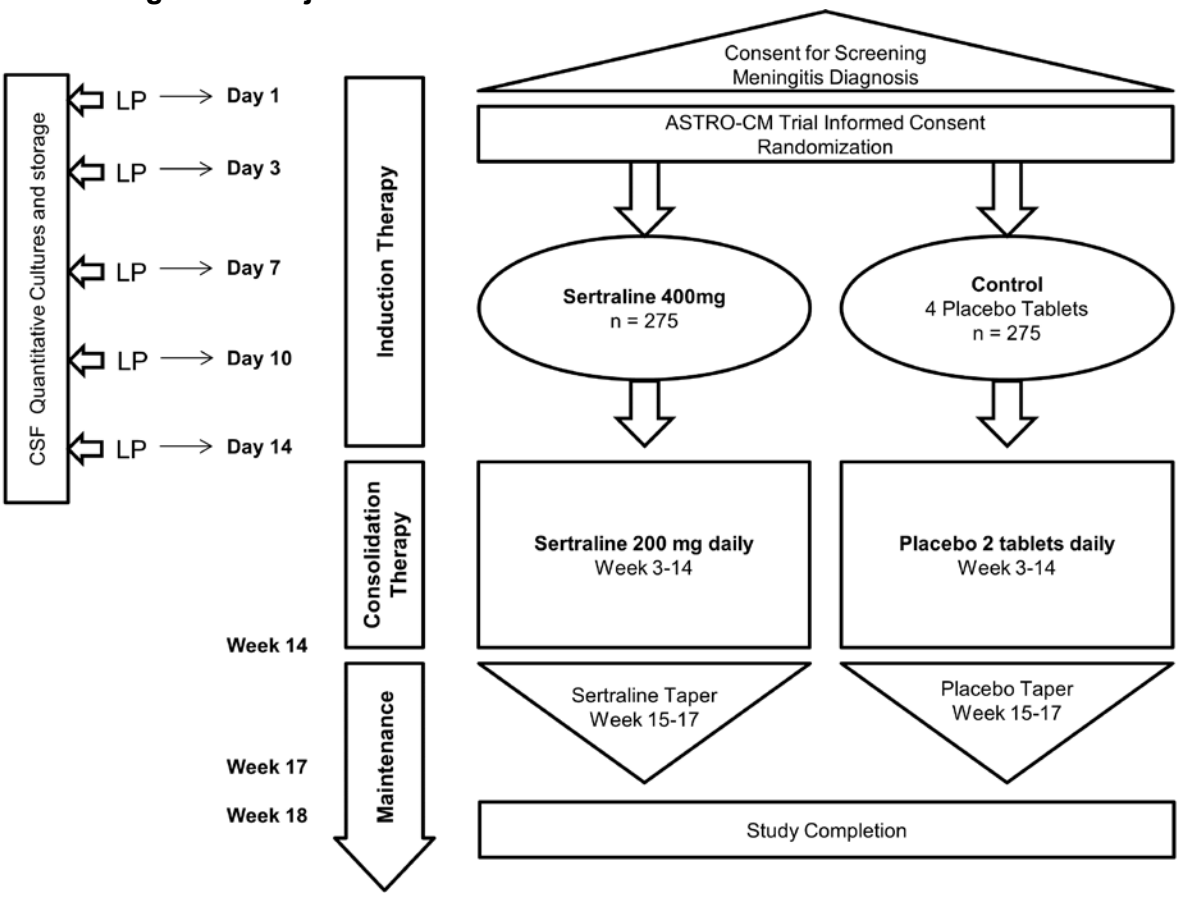
4 STUDY DESIGN

This is a phase III randomized, blinded and placebo controlled trial to evaluate the effect of sertraline when added to standard therapy for CM, with the hypothesis that adjunctive sertraline will lead to faster fungal clearance and improved survival. CM diagnosis will be made via CSF cryptococcal antigen (CRAG) at time of lumbar puncture (LP) with confirmation by CSF culture. After informed consent, subjects with CM that meet eligibility requirements will be enrolled into the study. A non-randomized phase I dose-escalation study was first conducted from August 2013 to August 2014 to help optimize dosing for this randomized phase III trial.

4.1 Randomized Trial Design

Subjects will be randomized to standard therapy per WHO guidelines with masked placebo or sertraline at 400mg/day (**Figure 5**). Dose of sertraline was chosen based on the results from the Phase I study.

Figure 5: Trial Design and Subject Flow



We will use a permutated block randomization in a 1:1 allocation (n=275 per arm). Subjects will receive sertraline at the 400 mg/day (four 100 mg tablets) or masked placebo (four tablets) throughout the 2-week induction period. CSF from the LPs at diagnosis, day 3, day 7, day 10, and day 14 will be sent to the microbiology lab for quantitative cryptococcal culture.[43] Quantitative cultures will be used to calculate early fungicidal activity (EFA) over 14-days, and we will compare EFA between sertraline vs. placebo.

Subjects may be discharged from the hospital upon completion of induction therapy, if they are ambulatory and their clinical condition has been deemed stable by the study medical officer. Fluconazole 800-1200 mg/day will be prescribed at hospital discharge.

At the end of 2 weeks, the dose of study drug will be decreased to 200 mg daily. Those randomized to sertraline will receive two 100mg sertraline tablets daily, while those randomized to the placebo arm will receive two placebo tablets daily. Study drug will begin to be tapered off starting after week 14. The taper entails decreasing the study drug to 100mg/day (one tablet of sertraline or placebo) for 2 weeks, and then 50 mg/day (one-half tablet of sertraline or placebo) for one week until off sertraline or placebo by the end of study week ~17. The tapered discontinuation is intended to avoid a withdrawal syndrome.[49] The adherence of exact dosing during the taper will not be assessed in individual participants.

ART will be initiated approximately 4-6 weeks after CM diagnosis per current WHO standard of care.[50] After ART initiation, subjects will transition to consolidation CM therapy with ~10 further weeks of fluconazole (400 mg/day). Outpatient visits will occur initially every 2 weeks at the study site outpatient HIV clinic until week 10 and then monthly until study conclusion of the study at 18 weeks. Assessment of neurocognitive function and depression symptoms will occur at 14 weeks. Patients can elect to continue to receive routine HIV care after study completion. If persons have active cryptococcal-related issues (e.g. IRIS or relapse) ongoing at 18 weeks, the participant will continued to be followed by the study team until their condition has stabilized.

4.2 Pharmacokinetic Studies

Sertraline and N-desmethylsertraline concentrations were measured on plasma and CSF from patients enrolled the Phase I pilot study (Aug 2013- Aug 2014). A range of time points was selected for each oral dose to evaluate how rapidly sertraline comes to steady state.

In the phase III trial, amphotericin, fluconazole, and sertraline concentrations will be measured in day 7 and day 14 plasma or CSF samples for a subset of participants, to help understand the interaction between sertraline and standard induction therapy.

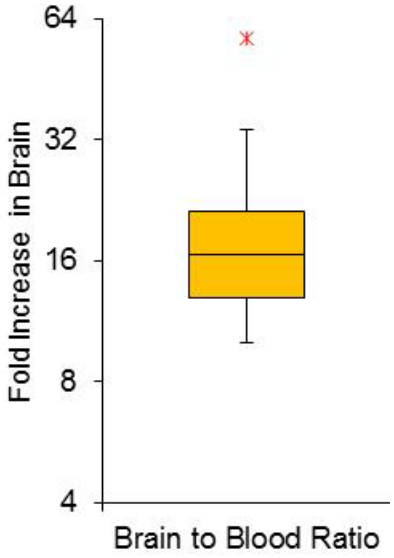
All PK studies will be performed in the Clinical Pharmacology Analytical Services Laboratory at the University of Minnesota, which has previous experience quantifying sertraline, amphotericin, and fluconazole levels in biologic specimens. The analytical method used is high performance chromatography–mass spectrometry (HPLC-MS), which requires at least 0.1mL of sample. This is a research assay, which does not have clinical utility at present. Thus, there is no plan to establish an analysis site in Africa.

4.3 *In vitro* Microbiology Studies

In vitro sertraline susceptibility assays will be performed on Ugandan *C. neoformans* isolates from the COAT trial (ClinicalTrials.gov Identifier: NCT01075152), initially to determine the target MIC being sought in the Phase I pilot study (Aug 2013). The MIC for sertraline in these isolates will be obtained according to Clinical and Laboratory Standards Institute (CLSI) standards. The minimum inhibitory concentration (MIC) of

fluconazole, alone and in combination with sertraline, will also be investigated. All in vitro susceptibility assays will be performed under the supervision of Dr. Kirsten Nielsen at the University of Minnesota. In preliminary experiments the median MIC is 4 μg/mL (IQR: 2-4) among 135 Ugandan *Cryptococcus neoformans* isolates with 81% of isolates having a MIC ≤4 μg/mL.

4.4 Dosing of Sertraline



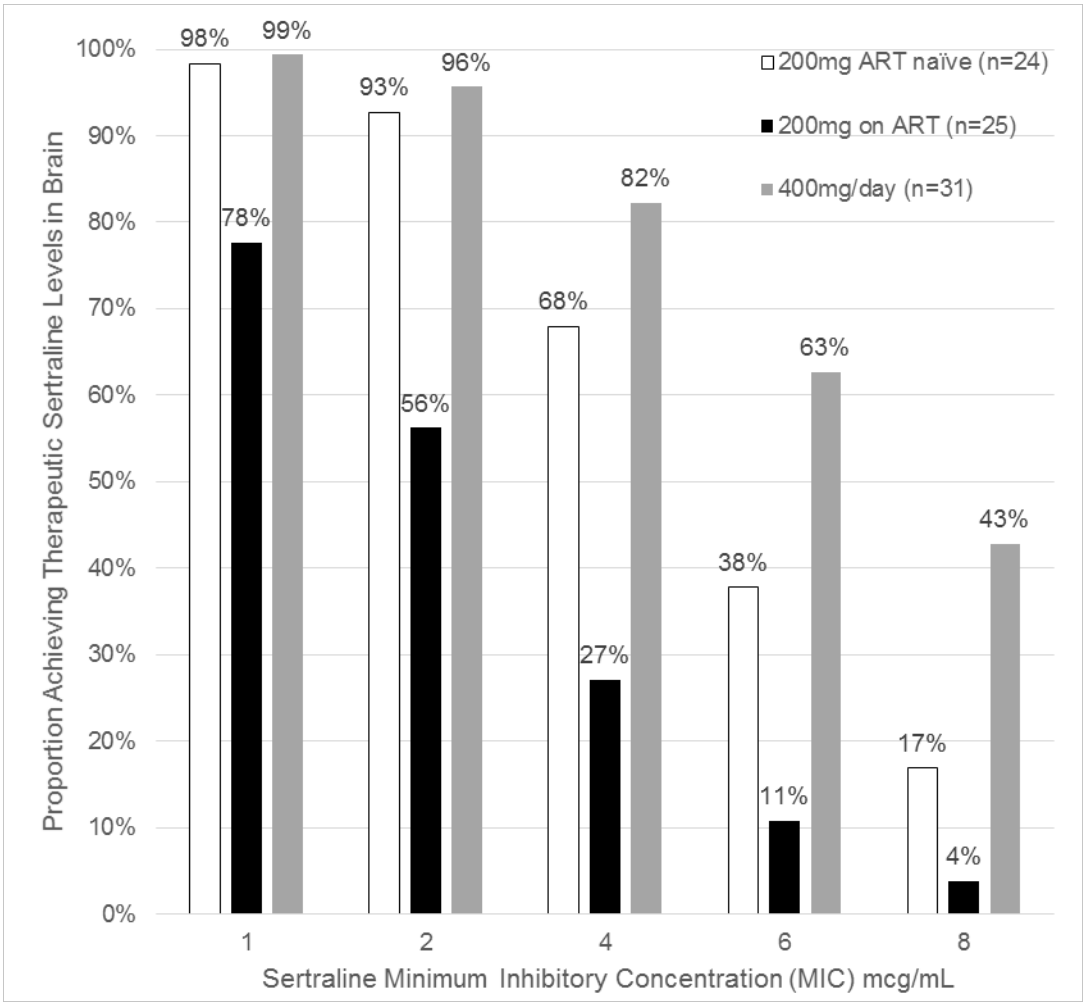
The sertraline dose selected for use in this trial is based on the following considerations:

1. 200mg/day of sertraline is the current maximum U.S. FDA approved dose for depression, and has an extensive tolerability and safety record. Thus longer-term use is safe.
2. *Sertraline levels observed in PK studies of CNS*. In a 2013, U.S. Federal Aviation Administration (FAA) analysis of sertraline concentrations in postmortem fluids and tissues in 11 aviation accident victims, the average fold increase in concentration in the brain was 21.7x that of blood concentrations (Figure right) with a median 16.5 (IQR: 13 to 21) fold increase.[51] The concentrations in the brain observed were 1.5 mcg/mL (IQR: 1.0 to 2.6).
   a. The dose of sertraline the airplane crash victims were receiving was not known; however the median blood concentration of 6.4 ng/mL was the same as the median 6.4 ng/mL concentration observed in plasma among the 100mg sertraline dosing group in Uganda during the phase I pilot PK study (Aug 2013).
   b. Thus a 2-4 fold increase in brain concentrations (using 200-400mg/day sertraline) puts projected median brain tissue concentrations for 200mg sertraline dose at 3.0 (IQR: 2 to 5) mcg/mL and for 400mg dose at approx. 6.0 (IQR: 4 to 20) mcg/mL. The 400mg dose has a high probability of being above 81% of the *Cryptococcus* MIC of 4 mcg/mL (IQR 2-4) from Ugandan isolates (Section 4.3 above), and may be clinically effective with the known *in vitro and in vivo* synergy with fluconazole.[3]
3. Sertraline rapidly achieves steady state concentrations with similar plasma levels observed between day 7, 10, and 14 in the phase I PK pilot (Aug 2013); however the 400mg/day loading dose achieves steady state above the MIC faster. Levels at Day 3 are ~83% of steady state levels at days 7-14.

Plasma Levels (ng/mL) in patients receiving 200 or 400 mg/day Sertraline
based on Phase I ASTRO pilot study (Aug 2013).

		Avg	Stdev			Avg	Stdev
Day	N	Sertraline 200 mg/day		**Day**	n	Sertraline 400 mg/day	
3	28	**162**	±104	**3**	25	**348**	±164
7	29	**240**	±172	**7**	20	**398**	±164
10	24	**271**	±192	**10**	19	**484**	±237
14	24	**217**	±224	**14**	14	**417**	±280

4. Expected Therapeutic Concentrations in Brain Tissue, based on Monte Carlo simulation modeling of the 25,000 simulations of the distribution of plasma drug levels; 2) CNS penetration into brain tissue; 3) *Cryptococcus* MIC susceptibility, is provide in Figure.



Based on the available data, antifungal sertraline steady state concentrations in the brain parenchyma are anticipated to be therapeutic in approx. 81% of participants using the currently proposed 400mg/day induction dosing scheme. With a two-fold fluconazole synergy/additive effect, 95% of participants would be expected to have a therapeutic level in brain. At 400mg/day of sertraline, there is minimal drug-drug interaction with efavirenz on the sertraline plasma levels.

5. *Evaluation of safety including frequency and severity of sertraline side effects*. Given the large therapeutic window and safety track record for sertraline, major side effects are not anticipated at effective antifungal drug concentrations. In the pilot study (Aug 2013), no dose limiting toxicity was observed with doses of up to 400mg/day for 14 days.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 Subject Inclusion Criteria

- Cryptococcal meningitis diagnosed by CSF cryptococcal antigen (CRAG)
- HIV-1 infection
- Ability and willingness of the participant or legal guardian/representative to provide informed consent
- Willing to receive protocol-specified lumbar punctures

5.2 Subject Exclusion Criteria

- Age < 18 years
- Receipt of ≥3 doses of amphotericin therapy
- Previous history of cryptococcal meningitis (may be followed on compassionate basis but will be excluded from randomization)
- Cannot or unlikely to attend regular clinic visits
- Presence of jaundice or known liver cirrhosis
- Currently receiving any anti-depressant medication at time of enrollment
- Pregnancy
    - If there is a concern of pregnancy, a negative urine (or serum) pregnancy test before study entry is required.
    - Women of childbearing potential will have pregnancy test within 48 hours of enrollment and will be recommended to use contraception and referred to family planning services as necessary. (Refer to informed consent document.)
- Currently breastfeeding

Rationale for Criteria

The inclusion and exclusion criteria are meant to be broad to enroll a representative sample of persons in resource-limited areas, and thereby be broadly generalizable. Fluconazole is a known teratogen in the first trimester and sertraline may put newborns at risk for the development of persistent pulmonary hypertension of the newborn (PPHN) when taken during the last half of pregnancy. Thus, pregnant and breastfeeding women are excluded. However, these risks are associated with long-term use of these medications, and a 48-hour period from time of enrollment to pregnancy test will be allowed to rule out pregnancy in women of childbearing age, unless it is specifically suspected based on history and exam. In this case, a pregnancy test is required prior to enrollment.

The main site of sertraline metabolism is the liver, and drug clearance of sertraline can be reduced and could be unpredictable in patients suffering from liver impairment. Therefore, patients with jaundice, suggestive of significant liver disease or cirrhosis, will be excluded from the trial. Other exclusion criteria are intended to minimize bias and maximize likelihood of completing the study.

Note on subjects currently receiving ART or TB treatment at time of enrollment

Based on limited observational studies, both efavirenz and rifampin are suspected to lower sertraline levels during co-administration.[52,53] However, since these medications are commonly used together in

patients with CM, clarifying the effect of these medications on drug levels during co-administration has important implications for the use of sertraline as an adjunctive agent. Therefore, these patients will be eligible to enroll in the study so that CSF PK data can be collected and interpreted in patients concurrently receiving treatment for HIV and TB.

In the phase I pilot study, there was a minimal apparent effect of ART on sertraline plasma concentrations with the average ratio of 93% (±19%) (Range 73-125%) in comparison of similar visit and doses between measurements of specimens from persons receiving ART (n=48) and not receiving ART (n=46).

Though subjects currently receiving ART will be eligible for enrollment into the trial, randomization will be stratified by ART use. For the purposes of stratification, ART use will be defined as either,

- ***Receiving ART***: currently receiving ART at time of study enrollment or has received ART at any time during last 30 days prior to study enrollment, or
- ***Not receiving ART***: has not received ART at any time during last 30 days prior to enrollment.

Further PK analysis may provide insight on the interaction between rifamycin (rifampin), isoniazid, and sertraline, and study drug dosing may be altered in the future, via a Memo of Clarification to study investigators, which will be submitted to IRBs. At present, there is "insufficient clinical evidence to definitively establish the potential for an adverse interaction between isoniazid and antidepressants."[54]

Note on subjects with previous history of cryptococcal meningitis

Subjects with a previous history of prior, known cryptococcal meningitis will be provided sertraline on a compassionate basis, though they will be excluded from enrollment in the trial, randomization, and primary analysis. The rationale for providing compassionate use of sertraline in this patient population is that there is a strong association between disease relapse and fluconazole resistance,[2] and we therefore believe that the addition of sertraline could be beneficial in this patient population. The experience of persons in Uganda with cryptococcal relapse with fluconazole resistant *Cryptococcus* is near 100% mortality (unpublished data). Patients with a known previous history of CM that are followed by study personnel will be consented for sertraline administration and treated according the best clinical judgment of the study medical officer. In practice, for example, it is impossible to differentiate CM relapse from Immune Reconstitution Inflammatory Syndrome (IRIS) until CSF cultures have returned, which can take up to 10 days. In these cases, the medical officer may decide to treat initially as relapse disease, but may take an IRIS-specific approach to therapy (corticosteroids, abbreviated induction course, etc.) if there is high enough suspicion.

Patients with confirmed microbiological evidence of relapse disease will be consented and followed by the study team, and will receive open-labeled sertraline at 200-400 mg/day through the duration of the study. Clinical information will continue to be collected on these patients as long as they are receiving sertraline. If CSF cultures on initial lumbar puncture remain negative after 10 days, however, study drug will begin to be tapered off over a 3-week period, and the patient will be followed only as long they continue to receive study drug. In these cases, the study team will continue to follow the patient while in the hospital, and for 2-4 additional outpatient visits. Once the subject has completed receiving study drug, they will be referred back to their primary HIV provider, and will no longer be followed by the study

personnel. The reason for non-inclusion in the study will be clearly stated (i.e. history of previous CM). Contact information will be provided to the patient in case any further questions arise.

Note on subjects with positive CSF CRAG but negative CSF culture

Since CSF CRAG appears to be more sensitive than CSF culture in very early disease with a low fungal burden,[9] CSF culture-negative patients with no previous history of CM but a positive CSF CRAG will be assumed to have CM and will still be eligible for the study. Subjects with a positive CSF CRAG but with a negative CSF culture may be suffering either from early primary CM (ART naïve and no history of previous cryptococcal disease) or paradoxical CM-IRIS (if receiving ART and have history of previous CM). Patients with no previous history of CM will be eligible to enroll in the trial, while those with a previous history of CM will be consented to receive sertraline and will be followed clinically though not enrolled in the trial, as outlined above.

5.3 Treatment Assignment Procedures

5.3.1 Selection of Study Population

Potential research participants will be identified by active surveillance during hospitalization by the onsite study teams. Secondary surveillance will be via the site microbiology laboratories with study team notification of new positive culture or CRAG results.

5.3.2 Timing of Study Enrollment

Active recruitment into the study will occur from 9am-4pm, Monday to Friday. Potential subjects identified outside of these hours will still be eligible to enroll in the study, though enrollment may be delayed until facilities (clinical and microbiology labs) are open and the full study team is available to perform study procedures related to subject screening, enrollment and randomization. Receiving treatment for CM (initiation of amphotericin, lumbar punctures, etc.) is not dependent on enrollment into the study and will be started promptly upon identification of CM patients regardless of the time. Enrollment may be delayed for up to 2 days after starting amphotericin. Persons who have already received 3 or more completed doses of amphotericin will not be eligible for the study.

5.4 Informed Consent Process

5.4.1 Screening Consent

A screening consent (or surrogate consent from caregiver/next of kin) will be required for study personnel to perform a screening lumbar puncture (LP) for CM diagnosis to verify inclusion criteria for the trial. This consent also includes an optional specimen storage.

Persons referred from health care facilities outside of the study site with appropriate documentation of CM per study inclusion criteria will be eligible for the trial. However, these persons must still have an LP with full diagnostic CSF analysis performed as part of the study (after screening consent and prior to entry) to exclude alternative diagnoses.

5.4.2 Informed Consent

Informed consent is a process that is initiated prior to an individual's agreeing to participate in the study and continuing throughout the individual's study participation. After CM diagnosis and eligibility criteria

are confirmed, a study investigator will obtain full informed consent for the study by providing potential subjects with an approved consent form in English, Luganda (Kampala), Runyankole (Mbarara), or Swahili (Ifakara). As the study drug is integral to induction therapy, informed consent must occur within 2 calendar days of the potential study participant receiving their first dose of amphotericin. However, potential participants will be given appropriate time to consider the study. At the time of informed consent, a complete history and physical examination will be performed to verify inclusion/exclusion criteria (Section 5.1 and 5.2).

During the Informed Consent process, the investigator will describe the purpose, risks, and benefits related to the study. Each aspect of the forms will be explained in detail with the potential subject, and the potential subject will have the opportunity to ask any questions that he or she may have about the study. The investigator obtaining informed consent will ask questions to assess the subject's understanding. The investigator will state that participation is voluntary and that subjects may refuse participation or withdraw at any time without prejudice to their clinical care. Persons who decline participation will continue CM induction therapy according to current standards of care.

Since this study may require additional blood draws and biological samples beyond the standard of care, additional consent will be required from subjects receiving blood draws or other sample collection for stored samples for future study purposes. In this case, samples will be stored indefinitely, unless the subject has a change of mind and asks for them to be destroyed.

Alternatively, subjects may give limited consent to the collection, testing and storage of samples (blood, spinal fluid, and urine) for the purposes of this research only. This does not require additional blood draws, and all samples will be destroyed when the study is completed. Patients giving this limited consent will still be able to participate in the study.

An alternative informed consent form will be provided for patients that have a previous history of CM. As outlined in Section 5.2, these patients are excluded from the trial, but will still receive open-labeled sertraline on a compassionate basis.

If, in the opinion of the investigator, potential participants do not have appropriate comprehension, the investigator must re-explain the study or determine whether the participant's current mental capacity is diminished due to the pre-existing CM. If the potential subject is deemed in the opinion of the investigator to be unable to give informed consent, the investigator may choose to delay informed consent or pursue surrogate consent. If surrogate informed consent is pursued, the potential subject should be involved in the conversation along with the caregiver (giving the surrogate consent).

Consent for Lumbar Punctures (LP): The lumbar puncture procedure will be explained in detail in the informed consent form and patient information sheet. All LPs will require verbal informed consent. Refer to Section 8.2 for the recommended schedule of LPs.

Storage Consent: Informed consent for long-term storage of samples for future research testing is necessary by Ugandan regulations, and this will be included in the consent process. Refer to Section 13.5 for further details. In Tanzania, specimen storage will not occur beyond the study duration.

5.4.3 Surrogate / Proxy Consent

Subjects unable to give informed consent due to altered mental status may have surrogate consent obtained by proxy from their caregiver/next of kin. An estimated ~30% of the study population will likely have altered mental status at initial CM presentation.[9]

Comprehension of the informed consent process should be assessed in persons with near normal mental status (GCS 15). If, in the opinion of the investigator, potential participants do not have an acceptable level of comprehension due to altered mental status, the investigator should pursue surrogate consent from the participant's legal proxy or not enroll the patient.

5.4.4 Literacy

A person who speaks and understands the language of the informed consent document, but does not read and write, can be enrolled in a study by "making their mark" via a thumbprint on the informed consent document.

In this event, an impartial, literate third party must witness the entire consent process and sign the informed consent document. The witness's name, signature, and relationship must be recorded on the informed consent document. A member of the study team is not an impartial third party.

5.4.5 Waiver of informed consent

CRAG LFA screening by fingerstick, serum, and/or plasma is to be performed as routine diagnostic care (without written informed consent) due to the high prevalence of CRAG+ individuals hospitalized as recommended in the Uganda National ART guidelines, December 2013. In Kampala during 2010, the prevalence of CRAG+ individuals with CD4<100 was 21% on the Mulago Hospital ward 4A.[55]

5.5 Randomization

Subjects enrolled into the study will be randomized at study entry. **The preferred time for randomization is the day of diagnosis.** Subjects must consent and enroll into the study before their third dose of amphotericin. A computer generated permutated block randomization algorithm will randomly determine treatment assignments at a 1:1 ratio. Randomization will be stratified by ART-use and clinical site.

Randomization schedules will be supplied to the pharmacy at each site, with notations for which dose to take during the induction phase and which study drug to take during the outpatient phase. Only the central pharmacy at IDI and the study biostatisticians will have access to both the participant study ID and the randomization group. Detailed instructions for study drug processing, labeling and dispensing are included in the ASTRO-CM Pharmacy Randomization and Drug Processing SOP.

5.6 Blinding

The trial will be a double-blinded study. Study medications will be pre-packed and blinded by a central pharmacist or designee assigned to the study. As sertraline has an extensive safety record, there will not be unblinding; however, participants may be switched to open label at clinician discretion if they are diagnosed with severe depression after week 4. For persons with perceived intolerance or toxicity, dose reduction may occur at physician discretion.

5.7 Study Withdrawal

A study subject will be discontinued from participation in the study if:

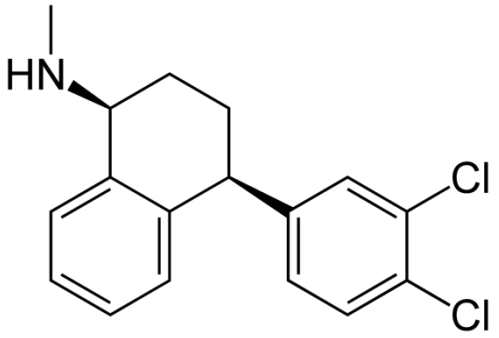
- HIV-negative.
- Any clinical adverse event (AE), laboratory abnormality, concurrent illness, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject.
- Subject wishes to voluntarily withdraw.

Subjects are free to withdraw from participating in the study at any time upon request. Every effort will be made to undertake protocol-specific study procedures. If voluntary withdrawal occurs, the subject will be asked to continue scheduled evaluations, complete an end-of-study evaluation, and be given appropriate care under medical supervision until the symptoms of any AE resolve or the subject's condition becomes stable. The patient will be offered ongoing follow-up care at the site outpatient HIV clinic or referred to a clinic of their choice if withdrawn from the study. If a subject is voluntarily terminating antifungal therapy, the site PI must be notified immediately, as the result will be near 100% mortality.

6 MEDICATIONS TO BE USED IN THE TRIAL

With the exception of the investigational drug sertraline used in this study, all medications received in this study are considered standard of care. Sertraline and/or placebo will be provided by the study. No other medications will be provided by the study.

6.1 Sertraline Description



Sertraline is a selective serotonin reuptake inhibitor (SSRI) for oral administration. Sertraline is supplied as scored tablets containing sertraline hydrochloride equivalent to 25, 50 and 100 mg of sertraline. Section 2.5.1 outlines the pharmacokinetics of sertraline.

Ample clinical data supports the safety of sertraline at doses between 50-200mg.[34-36] Few drug-drug interactions of clinical significance exist[33], and it is relatively safe in over-dosage.[37,38] Common adverse effects include nausea (25% vs. 11% for placebo), male ejaculation failure (14% vs. 1% for placebo), insomnia (21% vs. 11% for placebo), diarrhea (20% vs. 10% for placebo), dry mouth (14% vs. 8% for placebo), drowsiness (13% vs. 7% for placebo), dizziness (12% vs. 7% for placebo), tremor (8% vs. 2% for placebo) and decreased libido (6% vs. 1% for placebo).[56] These adverse events are often transient and resolve spontaneously without dosage adjustment. Sertraline also appears to be associated with microscopic colitis, a rare condition of unknown etiology.[57] Akathisia caused by sertraline was observed in 16% of patients in a case series.[58] Akathisia typically begins several hours after the initiation of treatment or a dose increase and usually disappears after being stopped or decreased. Prolonged QT interval is considered a class effect seen with SSRIs, particularly with citalopram. However, of the SSRIs, sertraline has one of the least effects on QT interval.[37] Nevertheless, the QT interval was monitored in all patients enrolled into Phase I open label pilot, via serial (weekly) EKGs from baseline through the day 14 visit.

In healthy volunteers over a 2-week period, sertraline slightly improved verbal fluency but did not affect word learning, short-term memory, vigilance, flicker fusion time, choice reaction time, memory span, or psychomotor coordination.[59] No clinically relevant differences were observed in objective cognitive performance in a group of people treated for depression with sertraline for 1.5 years as compared to healthy controls.[60]

All antidepressants, including sertraline, carry an FDA black box warning stating that antidepressants may increase the risk of suicide in persons younger than 25 years. This warning is based on pooled analyses of short-term placebo-controlled trials of antidepressant drugs (SSRI and others) that found a 1.5-fold increase of suicidal behavior in young adults (aged 18-24) with major depressive disorder.[61] Considered separately, sertraline use in adults decreased the odds of suicidal behavior with a marginal statistical significance by 37% or 50% depending on the statistical technique used.[61]

The development of a potentially life-threatening serotonin syndrome has been reported with SSRIs, including sertraline, alone but particularly with concomitant use of other serotonergic drugs. The concomitant use of sertraline with the MAOI class of psychiatric drugs is contraindicated.

Abrupt interruption of sertraline may result in withdrawal or a so-called discontinuation syndrome. This syndrome occurred in 60% of subjects in a blind discontinuation study where sertraline was temporarily replaced by placebo.[62] Frequent symptoms reported include irritability, agitation, dizziness, headache, nervousness, crying, emotional labiality, bad dreams and anger. This withdrawal syndrome was completely avoided when sertraline was gradually discontinued over three weeks.[49]

Acquisition: Sertraline is available locally in Uganda and Tanzania. Its approval for use in study was granted by the Uganda National Drug Authority, and Tanzania Food and Drugs Authority. A U.S. FDA-approved sertraline manufacturer will be used.

Product Storage and Stability: Sertraline will be stored at room temperature (25ºC with excursions permitted to 15ºC-30ºC) according to manufacturer guidelines.

Study administration: Patients enrolled in this study will receive sertraline or placebo, as outlined in Section 4. This trial will use sertraline 100 mg tabs or masked placebo (**Table 5**). The use of sertraline (or placebo) is divided into three phases. During the induction phase (the first 2 weeks), subjects will receive the experimental 400mg dose of sertraline as determined in the study design. After receiving 14 days of sertraline at the experimental dose, all subjects enrolled into the sertraline arm will change to a dose of sertraline 200 mg/daily, and subjects in the control arm will continue to receive placebo. Sertraline (or masked placebo) will begin to be tapered off starting after week 14. The taper will entail taking 100mg (one tablet) daily for 2 weeks and then 50 mg (one-half tablet) daily for one week. Participants will be off of all study drugs for the final week of the study (week 18). The tapered discontinuation is intended to avoid a withdrawal syndrome.

Table 5: Schedule of study drug received during ASTRO-CM trial

Randomization Group	Induction Weeks 1-2	Consolidation Weeks 3-14	Taper Weeks 15-16	Taper Week 17	Taper Week 18
Placebo Control	4 Placebo tabs	2 Placebo	1 Placebo	0.5 Placebo	None
400mg Sertraline	4 Sertraline tabs	2 Sertraline	1 Sertraline	0.5 Sertraline	None

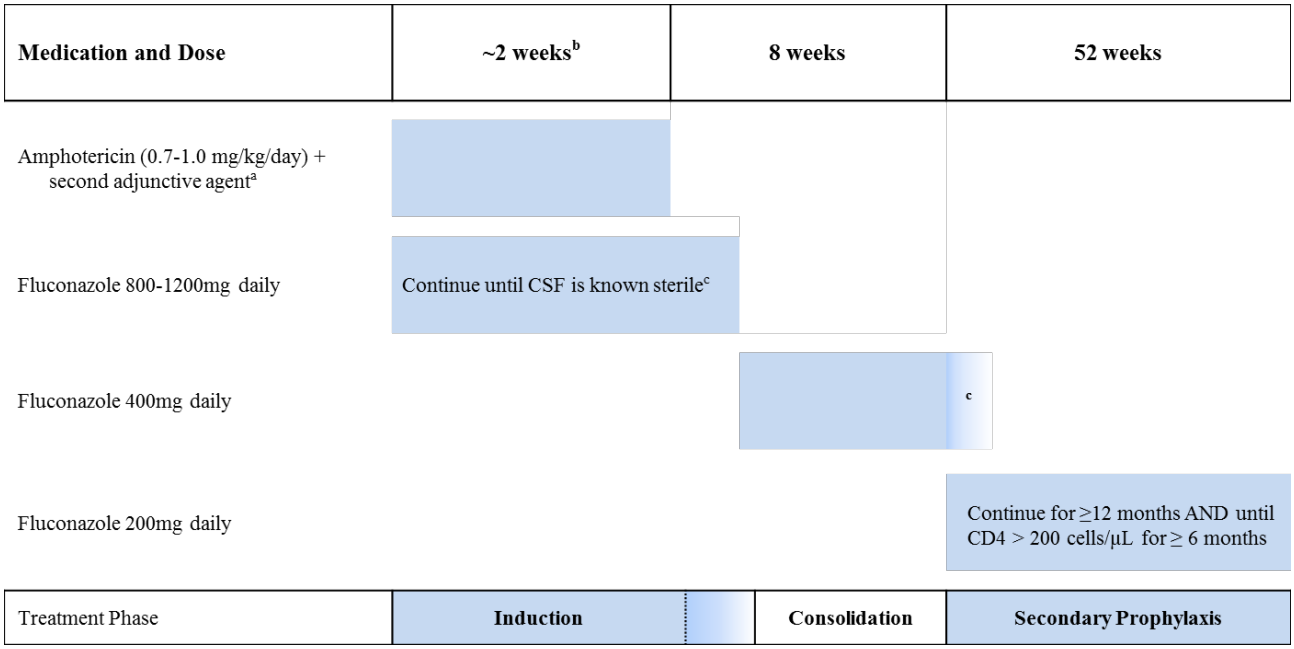
Drug-drug interactions: There are no drug interactions from sertraline with amphotericin, fluconazole, or first-line ART, with the exception of efavirenz, which is reported to lower serum sertraline levels by 39%.[52] For ART-naïve persons, efavirenz will not be started until 4-6 weeks. For persons on ART at randomization, potentially lower sertraline levels may occur. This is additional rationale for stratifying randomization by ART-status and for testing higher than “traditional” doses of sertraline, which once metabolized will be potentially in the high-normal range. For persons newly starting ART at 4-6 weeks after cryptococcal meningitis diagnosis, the induction of sertraline metabolism by EFV will assist with tapering of the medication to prevent any side effects from abrupt discontinuation. With the exception of sertraline or placebo, ongoing management of CM and initiation of ART will be according to accepted international guidelines. It has been similarly theorized that the anti-TB medication rifampin similarly induces sertraline metabolism, leading to decreases in plasma concentration, though this is based on limited observations.[53]

6.2 Permitted Medications Following CM Standard of Care

6.2.1 Cryptococcal Meningitis Management

Standard CM medication management in resource-limited settings is summarized in **Table 6.** This is the recommended therapy; however, this study will utilize the local standard of care based on medication availability. Consolidation therapy will be extended for an additional 12 weeks (i.e. study week 14).

Table 6: Recommended Treatment for Cryptococcal Meningitis in Resource-Limited Settings



Notes: [a] Flucytosine (5FC) 100 mg/kg/day preferred where available, otherwise fluconazole at 800–1200mg/day in divided doses. KCl 40–60 mEq/day should be given amphotericin; [b] optimal duration of initial induction therapy is unknown. In resource-limited regions, the cost-benefit is likely maximal for one week induction with amphotericin B 1 mg/kg/day coupled with 2 weeks of fluconazole 1200 mg kg/day as the induction; [c] consider longer duration of induction and consolidation therapy if CSF culture positive at 2 weeks.

Individual patient factors often alter patient care and fluconazole dosage on an individual basis at physician discretion, such as acute renal failure, duration of cryptococcal culture positivity, CM relapse, medication intolerance, and hepatic P450 drug-drug interactions (e.g. rifamycin).

The major difference between the *standardized CM management* and the IDSA or DHHS guidelines is that flucytosine (5-FC) is not available in this setting and is not given in combination with amphotericin B induction.[13,63] If amphotericin is unavailable, high dose fluconazole 1200mg/day is recommended.

As well, some individuals will remain CSF culture positive after 14 days of induction antifungal therapy, thus higher dose fluconazole with fungicidal activity (800-1200 mg/day) will be used until outpatient clinic enrollment at Study Week 4. Thereafter, the fluconazole will be decreased to 400 mg/day at physician discretion in order to minimize pill burden and potential adverse reactions. For persons with persistently positive cultures, refer to Section 7.4 on outpatient clinical management of CM. Induction

therapy using a combination of amphotericin and high-dose fluconazole is recommended as the microbiologic activity is greater than that of amphotericin alone. Subjects who remain CSF culture positive should continue on at least induction-dose fluconazole until culture negative. **Table 7** summarizes the clinical management in regards to timing of negative CSF fungal cultures and dose reduction of fluconazole in persons who are clinically doing well.

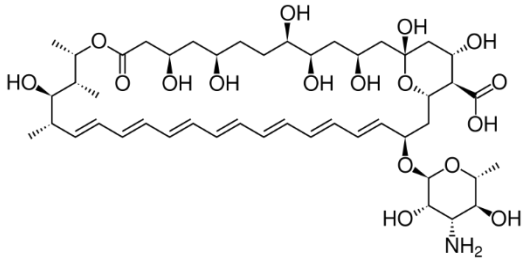
Table 7: Timing of CSF Cultures in Relation to Recommended Dose Reduction of Fluconazole

Study Visit	CSF Culture Status at Visit	Next LP	Fluconazole dose	Reduce Fluconazole Dose at Visit
Day 14	Negative	If symptomatic only	800-1200 mg	Week 4
	Positive	Week 4	800-1200 mg	After next LP if CSF neg.
Week4	Negative	If symptomatic only	400 mg	Week 14
	Positive	Week 6-10	800-1200 mg	After next LP if CSF neg.
Week 6-10	Negative	Do Not Repeat	400 mg	Discuss with Site PI, Week 14-18.
	Positive	Week 14	Discuss with Site PI, consider amphotericin re-induction	

Refer to LP management (Section 8.2) and outpatient CM management (Section 7.4) for details.

6.2.2 Antifungals Used in the Treatment of CM

6.2.2.1 Amphotericin B



Amphotericin B is an antifungal polyene antibiotic that is available in single vials. Each vial contains a sterile, non-pyrogenic, lyophilized cake providing 50 mg amphotericin B and 41 mg sodium deoxycholate buffered with sodium phosphates. Amphotericin B is solubilized by the addition of sodium deoxycholate to form a mixture, which provides a colloidal dispersion for intravenous infusion following reconstitution.

Typical dosing for CM is 0.7-1 mg/kg daily. Amphotericin should be administered by *slow* intravenous infusion over a period of approximately 4 to 6 hours (depending on the dose). The recommended concentration for intravenous infusion is 0.1 mg/mL (1 mg/10 mL).

Amphotericin is well known for its frequent and potentially severe side effects, as detailed in Section 2.3. For this reason, it is used only for the treatment of life-threatening fungal disease, such as CM, where it may be the only effective treatment available. In all cases, possible life-saving benefits of amphotericin must be balanced against its untoward and dangerous side effects.

Acquisition: In Uganda, amphotericin will be obtained from the Mulago Mbarara Teaching Hospital's Joint AIDS Program (MJAP), which receives amphotericin through PEPFAR as an

allowable expense for AIDS-related care. Any nationally registered or FDA approved formulation is acceptable.

Liposomal and lipid complex preparations of amphotericin B are rarely available in Africa, but may be substituted for amphotericin deoxycholate depending on availability and clinical circumstances. Only standard doses of commercially-produced, FDA approved and nationally registered formulations will be used in such situations.

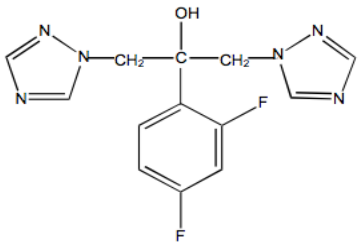
Product Storage and Stability: Prior to reconstitution, amphotericin should be stored under refrigeration 2° to 8°C (36° to 46°F) and protected against exposure to light.

Study administration: Subjects enrolled in this trial will typically receive standard up to 14-day courses of amphotericin B though duration may vary, according availability and to currently accepted guidelines. The 10-week survival (72%) observed during a trial in Mbarara, Uganda, following 5 days of amphotericin given with fluconazole 1200mg/day was quite similar to outcomes with 14 days of amphotericin.[64] Amphotericin is well known for its frequent and often severe side effects, as outlined in Section 2.3. Significant infusion reactions must be anticipated with a tendency toward IV-associated phlebitis. To help prevent this, IV lines will be flushed after amphotericin is administered and peripheral IV sites rotated at least every 3 days. Vigorous fluid co-administration of 1–2 L saline/day, along with careful monitoring for renal dysfunction and particularly electrolyte abnormalities (K and Mg) will also be carried out in this trial (Section 7). Electrolyte management with amphotericin is essential, and we will follow World Health Organization treatment guidelines including supplemental potassium and magnesium in increasing amounts during the second week of therapy.

Amphotericin induced renal insufficiency is expected; however data from an ongoing observational cohort indicates by the time of the week 4 visit, 80% have a creatinine $\leq$1.5 mg/dL and 96% $\leq$2 mg/dL (n=119).[9] At the discretion of the site investigator, the Manual of Operations (MOP) may be consulted for dose adjustment of ART with ongoing renal insufficiency with creatinine clearance (CrCl) <30 mL/min.

Guidelines for use of amphotericin B and management of common toxicities associated with amphotericin are included in the MOP and may be consulted, at the site investigator's discretion. Participants who permanently and prematurely discontinue amphotericin because of an AE will be followed closely until resolution of the AE can be documented.

6.2.2.2 Fluconazole



Fluconazole is a synthetic triazole antifungal agent that is available in 200 mg oral tablets. Fluconazole is a white crystalline solid which is slightly soluble in water and saline. Diflucan tablets contain 200 mg of fluconazole and the following inactive ingredients: microcrystalline cellulose, dibasic calcium phosphate anhydrous, povidone, croscarmellose sodium, FD&C Red No. 40 aluminum lake dye, and magnesium stearate.

Typical dosing is: 200-1200 mg daily. Duration of dosage depends on severity of infection. The oral bioavailability of fluconazole is $\geq$90%, and CSF concentrations are ~100% of plasma levels.

Fluconazole is a highly selective inhibitor of fungal cytochrome P450 dependent enzymes. It is a potent CYP2C9 inhibitor and moderate CYP3A4 inhibitor. Thus, patients who are on fluconazole and other drugs metabolized through CYP2C9 and CYP3A4 should be monitored.

Acquisition: Fluconazole will be obtained from the Ministry of Health, which receives fluconazole from Pfizer, Inc. and/or via the Diflucan Partnership Program. Any nationally registered fluconazole supply is acceptable.

Product Storage and Stability: Fluconazole needs to be stored below 86°F (30°C).

Study administration: Fluconazole is an oral medication, used for the treatment of many fungal infections including CM. In this study, we will be using doses of 800-1200 mg/day for induction therapy and until sterilization of CSF can be documented, as outlined in **Table 4**. The dose can be reduced to 400 mg/day for consolidation therapy after negative cultures for *Cryptococcus* have been documented. It can be further reduced to 200 mg/day after 14 weeks for secondary prophylaxis, assuming there is no reason to continue high dose fluconazole (history of CM relapse, CM-IRIS, concurrent fungal infection, or other reasons per medical officer clinical judgment).

The justification for the increased dose of 800-1200 mg/day is based on recent studies that have shown that fluconazole is more potent and safely tolerated at higher doses of 1200 mg daily.[10,20,64-66] In a study from Mbarara, for example, Longley et al showed that fluconazole was more rapidly fungicidal when administered at a dosage of 1200 mg/day versus 800 mg/day.[10] The quantitative rate of CSF clearance increases as the fluconazole dose is increased, but it is unknown if there is any benefit of increasing fluconazole beyond 1200 mg/day. This is the subject of a current National Institutes of Health-sponsored AIDS Clinical Trial Group trial (ClinicalTrials.gov: NCT00885703). The results of this ACTG trial may be reported during the ASTRO-CM trial, in which case, the trial steering committee may provide further recommendations to sites based on the available data.

Possible side effects of the medication are rare, but include headache, rash, nausea, vomiting, diarrhea, and abdominal pain. Patients will be warned of potential side effects at study entry, and closely monitored for adverse events. It is recommended that fluconazole doses of ≥800 mg/day be divided into at least twice daily administration to decrease potential GI side effects (e.g. nausea).

6.3 Accountability Procedures for the Study Intervention

Clinical personnel used in the dispensation and administration of study drugs (sertraline and placebo) or antifungals involved in the study will adhere to Good Clinical Practice (GCP) guidelines. Pharmacies utilized in the study will maintain the supply and record keeping of all study drug and antifungal dispensing. Such tracking is required per the Pfizer Diflucan partnership program in the case of fluconazole, and MJAP/PEPFAR in the case of amphotericin. Compliance with the protocol will be assessed via ongoing quality assurance monitoring via the DataFax management system.

6.4 Assessment of Subject Adherence to Study Interventions

While subjects are hospitalized, study personnel (nurse or medical officer) will directly oversee administration of study medication (sertraline or placebo) to ensure compliance. All amphotericin doses will be administered and/or recorded in the hospital by the study nurses. Self-reported compliance and pharmacy records will be used to assess study drug and fluconazole adherence during outpatient

management, and study drug tablets will be intermittently counted at study visits and sick visits to verify self-reports of study drug adherence.

6.5 Concomitant Medications

1. Concomitant use of amphotericin B and tenofovir is often avoided due to the potential increased risk of nephrotoxicity. Given the importance of both these medications for treating HIV-infected patients with CM, and the limited availability of alternative options in Africa, concomitant use will be permitted, though patients will be closely monitored for renal functioning. Subjects who require extended courses (>14 concurrent days) of amphotericin after initiation of ART (CM relapse, cryptococcoma, etc.) should not receive these medications concomitantly.
2. Rifampin enhances the metabolism of concurrently administered fluconazole. When administered together, we will consider increasing the fluconazole dose by 50%. Rifampin may also enhance the metabolism of sertraline, as discussed in detail in Section 5.2.
3. There is an interaction between sertraline and efavirenz, whereby sertraline levels are reported to be lowered by 39% by efavirenz, without an effect on efavirenz.[52] In the pilot study, plasma sertraline concentrations were only ~10% lower in persons receiving ART. Refer to Section 6.1 for detailed discussion.
4. Co-administration of other drugs known to prolong the QT interval and which are metabolized via enzyme CYP3A4 such as quinidine are contraindicated in patients receiving fluconazole. If malaria treatment is necessary, an artesunate-based regimen will be used.

   Prolonged QT interval is considered a class effect seen with SSRIs, particularly with citalopram. However, of the SSRIs, sertraline has one of the least effects on QT interval.[37] Nevertheless, the QT interval has been monitored in patients in the pilot phase I study (August 2013), and no significant prolongation of the QT interval has been observed (average change -4.1 ms $\pm$ 5.6 ms with 400mg sertraline at day 14).
5. Zidovudine (AZT): Fluconazole increases C_{max} and AUC of zidovudine by 84% and 74%, respectively, due to an approximately 45% decrease in oral zidovudine clearance. The half-life of zidovudine was likewise prolonged by approximately 128% following combination therapy with fluconazole. Patients receiving this combination will be monitored for the development of AZT-related adverse reactions.
6. Protease Inhibitors: Fluconazole may increase the serum concentration of some protease inhibitors. Thus, it is not recommended to administer high dose fluconazole with protease inhibitors. Patients in our study will not be started on protease inhibitors while still receiving high-dose fluconazole. Patients entering the study while receiving protease inhibitors will be monitored closely for interactions with fluconazole.
7. Nevirapine: Fluconazole is known to increase the concentration of Nevirapine.[67] If administered together, participants will be closely monitored for nevirapine-associated adverse events. Medical officers will be encouraged to prescribe an efavirenz-based ART regimen if possible, given drug interactions between nevirapine and fluconazole. The fluconazole dose is expected to be 400 mg/day at the time of ART initiation. This dosage of fluconazole used in conjunction with nevirapine is safe and well tolerated. [68] Thus nevirapine is not contraindicated; however, efavirenz remains preferred.

7 STUDY SCHEDULE

7.1 Schedule of Events

		Enrollment ↓										
Study Day		Day 1	Day 3	Day 7	Day 10	Day 14	Week 4	Week 6	Week 8	Week 10	Week 14	Week 18
Visit Code		0.1	0.3	0.7	1.3	1.7	4.0	6.0	8.0	10.0	14.0	18.0
Treatment Phase		Induction						Consolidation			2° Prophy	
Location	Hospital	→	→	→	→	→						
	Clinic (outpatient)						→	→	→	→	→	→
Procedures	Screening Consent	X										
	Informed Consent	X										
	Lumbar Puncture	X	X	X	X	X	[a]					
	Study Med Dispensing	Box				Bottle						
Medications	Sertraline (or Placebo)	400 mg/daily	→	→	→	→	200 mg/daily	→	→	→	Taper to off	
	Amphotericin B [f]	→	→	→	→	→						
	Fluconazole [b]	1200 mg/daily	→	→	→	→	→	400 mg/daily	→	→	200 mg/daily	→
History	Complete History	X										
	Interval History		X[c]	X[c]	X[c]	X	X	X	X	X	X	X
	Assess Adverse Events		X[c]	X[c]	X[c]	X	X	X	X	X	X	
Exam	Complete Physical Exam	X										
	Focused Physical Exam		X[c]	X[c]	X[c]	X	X	X	X	X	X	
	Neurocognitive Performance						Kampala				Kampala	
	Depression Scale						X				X	
Clinical Labs	CSF Analysis	X	X[d]	X[d]	X[d]	X[d]	X[a]					
	CRAG, CSF	X										
	CRAG, Blood { Serum, Plasma, or Fingerstick	X										
	HIV Antibody Screen	X[e]										
	HIV Viral Load, on ART			On ART >3mo								
	CD4, T-cell Profile	X										
	Creatinine	X	X	X	X	X	X					
	Potassium	X	X	X	X	X	X					
	Liver Function Tests	X										
	Urine Collection	X		X								
	Urine Pregnancy Test	Females										
Research Labs	CSF Collection		X	X	X	X	X[a]					
	PBMC Processing (Kampala)	X					X		X		X	

a) CSF Analysis via lumbar puncture is dependent on timing of CSF culture sterility. Subjects who remain CSF culture positive require are recommended to have follow up LPs to document sterility, see Section 8.2 for details.
b) The dose of fluconazole is recommended to decrease from 800-1200 mg/day to 400 mg/day only after a sterile CSF culture has been obtained.
c) Occurs daily while in hospital, but will only be documented if status change or adverse event (AE).
d) Follow up "therapeutic" LPs will have a limited analysis with quantitative fungal culture performed. See Section 8.2 for details.
e) If no previous documentation of HIV infection
f) Electrolyte and creatinine monitoring can be discontinued when amphotericin is completed.

7.2 Enrollment

At the time of initial hospital presentation, participants presenting with suspected CM will be evaluated by the study team. An LP will be performed by the study team per standard of care for evaluation of a patient with signs of meningitis (Section 8.2). In some cases, an initial LP will be performed by non-study medical personnel prior to contact by the study team. If CSF obtained from this LP is still available for further testing, it may be used for study enrollment purposes after screening consent has been obtained from the patient.

Study staff will verbally screen for inclusion/exclusion criteria when introducing the patient information sheet at the start of the informed consent process. Diagnostic CSF analysis typically includes:

- CSF WBC cell count
- CSF Protein (*optional*)
- Quantitative fungal culture
- Cryptococcal antigen (CRAG) by lateral flow assay with CRAG titer
- CSF storage for PK studies and future immunology assays
- Other CSF diagnostic studies as clinically indicated.
    - If ceftriaxone has been received prior to the lumbar punctures, Gram's Stain and Bacterial cultures are not recommended as they have zero diagnostic yield in Kampala from 2006-2012.
    - India ink has 20% less sensitivity than CRAG test.
- For CRAG-negative persons, additional diagnostics will occur. Refer to Section 8.2.1 and CSF Lab SOP for specific details.

Documented CM will be defined as either CSF fungal culture positive for *Cryptococcus neoformans* and/or CSF CRAG positive. As trial enrollment will be within the first 48 hours after diagnosis, CRAG will be used as the inclusion criteria, as the CSF culture takes >48 hours. CSF analysis will be performed at the local approved microbiology laboratory. The purpose of the first lumbar puncture is to make the diagnosis of CM (an inclusion criterion) and collect baseline CSF parameters and CSF specimens prior to anti-fungal therapy. As with every LP in this trial, CSF will be sent for quantitative cryptococcal cultures and stored for potential future PK studies.

If participants meet all inclusion/exclusion criteria and complete the informed consent process by signing the informed consent document, they will be considered eligible for enrollment into the study. After being enrolled into the trial, subjects will be randomized and the study intervention (sertraline or placebo) can be initiated.

A physician assessment at this time will include:

- Complete medical history
- Vital Signs
- Complete physical examination.

The medical history to be assessed and documented on the Enrollment CRF includes the past/current HIV/AIDS-related OIs, including TB, review of systems, all medications, and any allergies. (Refer to Section 8 for assessment definitions.)

At enrollment, current signs/symptoms occurring must be recorded. All grade 4 adverse events must be recorded on the enrollment CRF, so that new events may be correctly assessed and documented on an ongoing basis. Grade 4 adverse events are commonly expected among participants at study entry and will likely include: fatigue, weight loss, anorexia, nausea, and neurologic (behavior/altered mental status/cognitive/headache) abnormalities.

Within ± 24 hours of enrollment, baseline laboratories will be collected for:

- Complete blood count (CBC)
- Chemistries (K, creatinine)
- CD4 T cell count
- Liver function tests (AST, ALT, total-bilirubin)
- Rapid HIV test, if not previously documented
- Blood Culture, optional

We expect that most patients that undergo screening will already have a positive HIV antibody screen, but if no previous documentation of HIV infection is provided, one will be obtained at this time. HIV screening in this case will be the rapid test or ELISA per the current hospital protocol of universal HIV testing of all hospitalized patients (i.e. opt-out). A CD4 count with T-cell profile, CBC, and metabolic panel is expected as part of the routine medical standard of care in newly diagnosed HIV patient.

Whole blood for PBMC isolation will also be collected in Uganda at enrollment for future immunology studies. In addition, urine may be collected on all patients able to give a specimen for future diagnostic studies. PAXGene RNA tubes (2.5mL) will be collected a baseline in Uganda.

For all women of childbearing age with a concern of pregnancy, a urine pregnancy test will be obtained. A negative urine (or serum) pregnancy test is required prior to study entry for any woman with any concern of pregnancy.

As soon as possible after enrollment, street geographical mapping to the home or latitude/longitude coordinates of the home and up to 3 telephone contacts will be obtained to facilitate assessment of the primary endpoint (if necessary) to minimize losses to follow up.

Study days are numbered starting from the first day of enrollment (Day 1 = the day the subject is enrolled). The format of the visit code will be 'xx.y', where 'xx' is the study week, and 'y' is the study day of the corresponding week. For example, day 1 of the study is the day the subject is enrolled, and will be denoted '00.1'. Day 10 of the study (1 week and 3 days) would therefore be denoted '01.3'. To be considered eligible for the trial, induction therapy (amphotericin and/or high-dose fluconazole) must be started within a 2-dose window of enrollment. Assuming induction therapy is given on consecutive days, amphotericin may be started anytime from 2 days prior to enrollment in the study, to study day 00.3.

Summary of events during enrollment visit:

- Diagnostic lumbar puncture with CSF analysis
- Confirmation of study eligibility
- Informed consent
- Complete history and physical
- Baseline labs
- Randomization with initiation of study intervention

7.3 Hospital Follow-up Visits

Daily study visits will occur in hospitalized subjects with a study medical officer assessing:

- Interval history
- Focused physical examination, directed by symptoms

- Medications and adherence (as appropriate)
- Adverse events (AEs), grade 4 or 5

These visits will be documented on days 7 and 14, or as adverse events occur. Laboratory monitoring occurs for the duration of amphotericin administration on days 3, 7, 10, and 14, and will include:

- Serum creatinine
- Serum potassium
- Lumbar puncture with limited CSF analysis

After amphotericin is discontinued, routine serum creatinine and potassium measurements are not required.

In contrast to the initial “diagnostic” LP and subsequent complete CSF analysis, all follow up "therapeutic" LPs will have a limited analysis with CSF cell count and quantitative fungal culture performed as discussed in detail in Section 8.2.

A window period of ±1 day is allowable on visit days 3, 7, 10 and 14 to accommodate weekends, when the local laboratory may be closed.

For persons receiving ART for >3 months, a HIV-1 viral load is recommended once between day 7-14 at physician discretion to exclude virologic failure of ART.

If Grade 4 (potentially life-threatening) lab abnormalities are present or develop during the course of the study, laboratory monitoring will occur with greater frequency for subject safety. The following will be analyzed on a more frequent basis if severe abnormalities are present:

- Creatinine: if values > 3 mg/dL (>265 μmol/L), Cr will be repeated at least daily until stable
- Potassium: if values ≤ 2.5 mEq/L or ≥ 6.5 mEq/L, potassium will be repeated immediately and at least daily until improving

After 14 days of receiving study drug (sertraline or placebo), all subjects will receive a standard dose of sertraline 200 mg/day (or equivalent placebo if in the control arm), as outlined in Section 4.

Patients can be discharged from the hospital upon completion of induction therapy if they are ambulatory and their clinical condition has been deemed stable by the study medical officer.

In the event of a prolonged hospitalization, the study team will continue to follow the patient daily in the hospital, with assessments, evaluation, and monitoring undertaken according to expected routine standards of care. If any subject is hospitalized at the time of their expected clinic follow-up visit (week 4, 6, 8, 10, 14 or 18), the study visit will occur while in hospital, with assessments and monitoring as outlined.

7.4 Clinic Follow-up Visits

7.4.1 Clinic Registration, Orientation, and ART Counseling

All subjects will be scheduled to return for outpatient clinic registration in 1-2 weeks post-hospital discharge for clinic orientation and ART counseling, as necessary.

A study nurse will call the patient to verify plans for attending clinic. If subjects are physically unable to attend clinic by week 6, a home visit will occur by study personnel or a driver will be sent to retrieve the subject if necessary (e.g. if they are physically unable to use public transportation and do not have private transportation). If there are health concerns (and specifically if the subject needs to be retrieved

due to poor functional status and/or inability to ambulate), a study physician visit will occur as clinically needed for a 'sick visit', in addition to the scheduled events, as described in detail below.

7.4.2 Outpatient Study Visits

Subjects will be seen in clinic every 2 weeks after discharge from the hospital up until week 10, and then monthly until week 18. The first outpatient visit will occur approximately 1 week after clinic registration. For subjects who remain hospitalized at week 4, the visit will occur in the hospital.

At the Week 4 visit, a medical officer or designee will assess:

- Interval history
- Review of medications and adherence (with pill counts intermittently performed)
- Vital signs
- Assess AEs
- Focused physical exam, directed by symptoms
- Laboratory evaluation:
  - Serum creatinine
  - Serum potassium
  - Lumbar puncture if day 14 is not sterile and consent is provided
- Additional Research labs
  - Whole blood for PBMC isolation – immunology studies in Kampala
  - Storage of excess serum/plasma.
- Consider starting the process for ART initiation if clinically stable

At the week 4 visit, the subject's general health, hydration status, and need for re-hospitalization will be assessed. The study personnel conducting the week 4 visits will be a study nurse and a study medical officer, preferably organized such that at least one of the study personnel would have seen the subject at the day 14 visit. If clinically indicated, the subject will initiate ART according to national guidelines.

In the event of a prolonged hospitalization or re-hospitalization, the schedule of events will continue on schedule with laboratory monitoring and procedures conducted in the hospital or at a home visit.

A lumbar puncture will be repeated only if most recent CSF analysis is culture positive (with subject verbal consent) at time of visit. If the most recent CSF culture is positive, then induction dose fluconazole (800-1200 mg/day) will be continued. If the most recent CSF culture is negative at this visit, however, the dose will be decreased to 400 mg/day, which is the standard of care for consolidation therapy.

During subsequent outpatient study visits, patients will continue to have an interval history, medication adherence check, vital signs, focused physical exam, and assessment for AEs. No laboratory monitoring will take place during these visits unless clinically indicated, with the exception of additional whole blood draws for PBMC isolation and potential urine collection (weeks 8, 14 and 18) in Kampala, and selective analysis of HIV viral load (week 8 only).

The dose of fluconazole used will depend on whether or not CSF sterility has been documented, as previously discussed. As such, an LP will only be obtained in week 6 if most recent CSF analysis was culture positive and patient is still receiving induction doses of fluconazole (800-1200 mg/day). If patient is already receiving fluconazole at consolidation doses (400 mg/day) and culture remains negative, refills will be provided during this visit.

7.4.3 Week 14 Visit

Fourteen weeks signifies completion of therapy for CM (2 weeks induction + 12 weeks consolidation). Unless there is a reason to continue high dose fluconazole (history of CM relapse, CM-IRIS, concurrent fungal infection, or other reasons per medical officer clinical judgment), fluconazole will be decreased to the secondary prophylaxis dose (200 mg/daily) according to current guidelines.

At the 14 week visit, patients will begin their taper of study medication (sertraline or placebo) over approximately 3 weeks (see Sections 4 and 6.1).

Neurocognitive assessment will be performed at the Week 14 visit, with an allowable visit window of ± 2 weeks.

7.4.4 Week 18 Visit

Week 18 is the final scheduled visit of the study. Assessments obtained during this visit are identical to other outpatient visits as outlined above.

At the end of this visit, a study termination CRF will be completed for 18-week study conclusion. The subject will be asked to complete an end-of-study evaluation. The patient will be offered ongoing HIV follow-up care at the site HIV clinic or referred to a clinic of their choice.

7.4.5 Sick Visits

In all subjects with suspected CM relapse, CM-IRIS, or other health concerns, an urgent physician's visit will be made. Patients will be given the mobile phone number of the site PI/study officer to make urgent visits on their appointment card. At sick visits, study physicians will perform:

- Interval History, specifically emphasizing review of systems and medication adherence
- Vital Signs
- Complete Physical Exam
- LP (if CNS symptoms)
- Radiologic investigation (as clinically indicated)

If there is clinical suspicion for meningitis due to CM-IRIS or CM relapse, an LP must be performed (with subject verbal consent). The clinical presentation of a culture positive meningitis relapse and IRIS is clinically indistinguishable, and an LP with complete CSF analysis is necessary to differentiate IRIS from CM relapse. Clinically, CM-IRIS has a classic phenotype with the basic triad of:

1. Treated CM with a clinical response,
2. Recently started ART, and
3. Recurrent, aseptic (culture negative) meningitis.

The primary diagnostic consideration is in excluding cryptococcal relapse in which the CSF culture would be positive.

After a prior response to CM treatment with resolution of symptoms, evidence suggesting clinical worsening or new signs/symptoms would commonly include increased headache with or without fever or neurological deficits associated with increased intra-cranial pressure, increased lymphocytic CSF pleocytosis and/or development of cerebral cryptococcal abscess by contrast CT scan.

For persons with seizures, loss of consciousness, or focal neurologic deficit (other than cranial nerve VI palsy), a head CT should be performed to exclude other intracranial pathology or contra-indication to lumbar puncture (if CT available). If a CT is not available, LP should be performed at physician discretion. Cranial nerve VI palsy is common (20%) with increased intracranial pressure >200 mm H_2O. The purpose of repeat LP will be to differentiate CM relapse as well as to therapeutically relieve elevated intracranial pressure when present. Diagnosis of non-CNS cryptococcal IRIS will be based on clinical signs (e.g. lymphadenopathy), imaging, and/or biopsy of the relevant site with histopathology and/or culture to exclude alternative etiologies (e.g. AFB).

All other non-IRIS or non-relapse clinical events will be in accordance with current guidelines and existing clinic practices. Refer to Section 9 on AEs and for additional information.

Any CSF obtained during sick visits will have complete CSF analysis (Section 8.2.1). Remaining CSF will be stored for PK studies and future immunology studies.

7.5 Termination of Study

At study termination, the reason for study termination will be documented. Reasons for study termination are 18-week study completion, withdraw of consent, death, transfer of care, or loss-to-follow-up. At the study termination visit, the following will be documented:

- Vital status;
- Interval history;
- New AEs and updates to / final outcomes for previously identified AEs;
- Whether or not study drug was discontinued early.
- Assessment of the adequacy of the blinding of the investigational medicine

7.5.1 Early Termination

In case of early termination, a reason for study termination will be documented. Reasons for early termination include withdraw of consent, death, transfer of care, or loss-to-follow-up. An attempt will be made to schedule a termination visit. Assessment and laboratory evaluations for an early termination visit will be the same as the week 18 visit.

If subjects withdraw from the study, they remain eligible for ART per national guidelines. Persons withdrawing will be encouraged to continue to receive ART from the outpatient clinic or referred to a clinic of their choice for ongoing HIV care and management of CM.

Subjects enrolled in the study but choosing to leave the hospital early against medical advice will continue to participate in the study if they wish. Additional phone calls by study personnel will encourage the subject to seek follow up HIV care and to rejoin the trial per the ongoing schedule of events. Assessment of vital status will continue via telephone calls at a minimum, unless consent is completely withdrawn.

Anyone voluntarily discontinuing study participation must have a specific request made to them at study termination of whether collection of vital status (dead / alive) via an 18-week visit or phone call is allowable.

For persons involuntarily defaulting from study participation due to circumstances beyond their individual control (e.g. moving residence due to financial reasons, etc), appropriate referral to local HIV-

care and a request for a week 18 visit will be made. Actual transport expenses may be reimbursed in these rare occasions with prior approval of the site PI.

8 STUDY PROCEDURES/EVALUATIONS

8.1 Procedures

8.1.1 Clinical History

8.1.1.1 Complete History

A complete medical history will review prior HIV history, TB history, other medical history, review of systems, medications, and allergies. In the case of altered mental status with the history obtained from a surrogate, the complete medical history will be repeated at the week 4 visit (or thereafter if ongoing altered mental status at 4 weeks).

8.1.1.2 Interval History

At each subject contact, investigators will seek information on adverse events by open-ended questioning and, as appropriate, by examination. This will include structured assessment of the interval history of the present illness, interval HIV OI history, review of systems, adverse events, new medications, and medication adherence. ART adherence should be assessed by self-report and unannounced pill counts.

All clearly related signs, symptoms, and abnormal diagnostic results will be recorded and grouped under one diagnosis. After entry, all signs/symptoms consistent with Grade ≥4 adverse events must be recorded at each study visit (refer to Section 9).

8.1.2 Physical Exam

8.1.2.1 Complete Physical Exam

A complete physical exam includes: vital signs, HEENT (head, eyes, ears, nose, and throat), neck, chest, cardiovascular, abdomen, extremities, skin, and neurologic exam, as well as GU exam as culturally appropriate.

8.1.2.2 Focused Physical Exam

A focused physical exam will be directed at current symptoms and complaints with vital signs and a targeted physical exam by study physicians at a minimum.

8.1.2.3 Quantitative Neurocognitive Performance Score (QNPZ-8)

Neurocognitive function testing will occur at week 14 visit using a quantitative neurocognitive performance (QNPZ-8) score.

QNPZ-8 is derived from a test battery, which includes:

- Grooved Pegboard test
- Color Trails 1 and 2 tests
- WAIS-III Digit Symbol test

- Finger Tapping test
- WHO-UCLA Auditory Verbal Learning Test
- Semantic Verbal Fluency test (category fluency)

Additional hearing and/or vision screening may be performed.

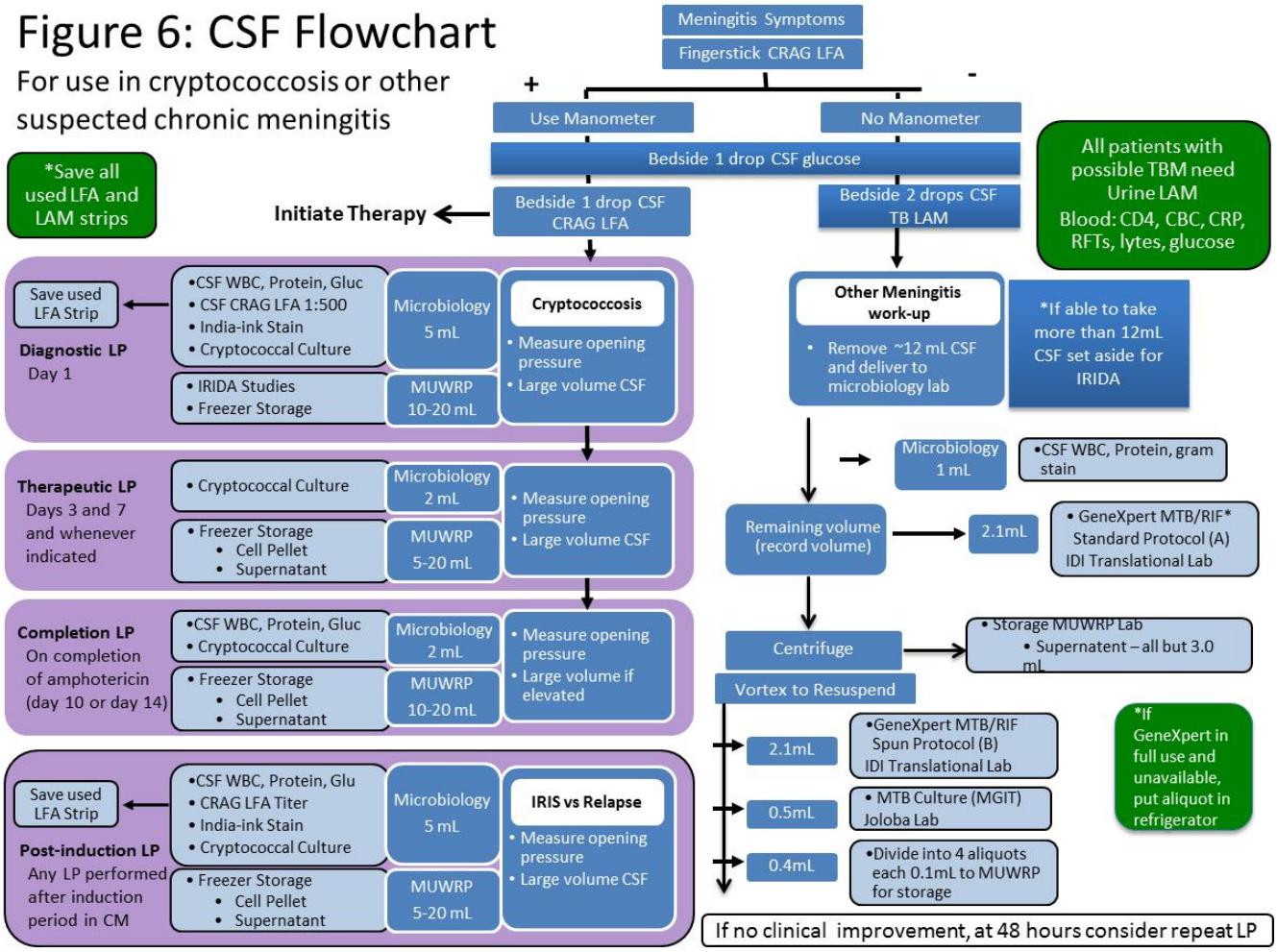
8.1.2.4 Center for Epidemiologic Studies in Depression (CES-D) scale

Depression will also be evaluated during weeks 4 and 14 using the Center for Epidemiologic Studies in Depression (CES-D) scale.[69] The CES-D consists of twenty items that are rated on a four-point scale (from 0: rarely or none of the time to 3: most or all of the time). Scores range from 0 to 60. Higher scores indicated a higher frequency of depressive symptoms during the last week.

8.2 Lumbar Puncture and CSF Analysis

8.2.1 Diagnostic Lumbar Puncture (LP) with Complete CSF Analysis

All patients with suspected meningitis will be screened for cryptococcosis by CRAG lateral flow assay (LFA). A CRAG LFA will initially be evaluated on whole blood obtained via fingerstick, with the results (return time = 10 minutes) directing subsequent work-up of CSF obtained by diagnostic LP (**Figure 6**). The screening informed consent process will be started after the fingerstick CRAG is collected. If the fingerstick CRAG is positive, a diagnosis of cryptococcal meningitis will be entertained and CSF studies will be directed to confirm this diagnosis. To evaluate for CM, a CRAG LFA will be performed on CSF obtained at the bedside during the LP procedure. Based on local infrastructure, study sites may customize CSF testing.



In cases of CM confirmed by bedside LFA, additional CSF studies will be obtained as outlined above. All LPs will be performed with manometers and have opening intra-cranial pressure (ICP) and closing pressure recorded. Initial microscopic exam will include WBC count with cell differential and India-ink stain. CRAG will be repeated at a titer of 1:500. CSF protein and glucose will be obtained. Quantitative fungal cultures will be performed. Remaining CSF sample will be stored at the Makerere University - Walter Reed Project (MUWRP) lab for future PK and pathophysiology studies.

If the screening fingerstick CRAG is negative, a diagnostic LP will be performed and non-specific CSF studies will be obtained to ascertain alternative diagnoses, according to standards of care and further outlined in **Figure 6**. Initial microscopic exam in this case will include AFB and Gram's stain. If no antecedent antibiotics have been administered, a bacterial CSF culture will be performed. Further evaluation for TB meningitis will include MTB culture and GeneXpert MTB/Rif on CSF. Refer to CSF Lab SOP for details. For CRAG-negative persons who have not clinically improved by ~3 days, a repeat diagnostic LP for repeat TB diagnostic evaluation should be considered, at physician discretion.

8.2.2 Therapeutic LP with Limited CSF Analysis

After cryptococcal diagnosis is known, follow up LPs will be performed to reduce ICP and document quantitative fungal culture to determine if the CSF is sterile. When an elevated opening CSF pressure is present (>200 mm), the therapeutic reduction of pressure is clinically indicated.[63,70] The differentiating feature between a 'diagnostic LP' and a 'therapeutic LP' is the CSF analysis performed on the CSF specimen. Follow up 'therapeutic LPs' will have a limited analysis, which includes CSF cell count, CSF protein, and quantitative fungal culture. A quantitative CRAG titer will be performed on stored specimens. Should a persistent headache recur anytime during the study, a therapeutic LP must be recommended to decrease the ICP. These follow up therapeutic LPs require verbal consent of the subject.

8.2.3 Quantitative Fungal Cultures

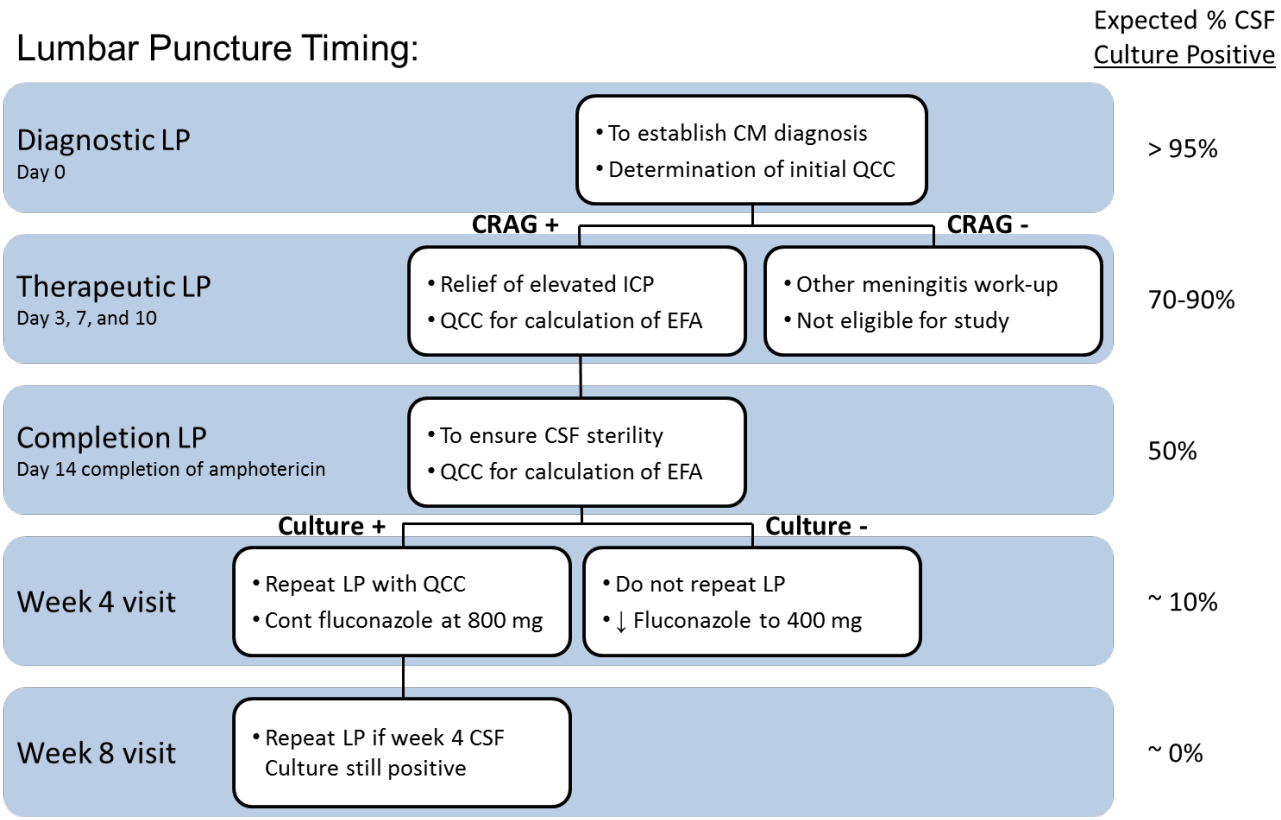
Fungal cultures will be performed per the SOP: "Protocol for quantitative CSF microbiology cultures for *Cryptococcus neoformans*" as provided by Dr. Tom Harrison.[43] The quantitative culture SOP includes an undiluted, standard CSF fungal culture, the result of which will be used for CM management. Quantitative counts will be performed by two separate lab technicians and counts averaged. Any cryptococcal growth on any dilution of the quantitative culture is abnormal and represents a positive CSF culture.

Cryptococcal isolates will be frozen at ≤ -20ºC in cryovials with 1mL of 25% glycerol. The purpose of storing isolates is to enable external quality assurance. External quality assurance will occur at the University of Minnesota, in the laboratory of Kirsten Nielsen Ph.D., to verify correct classification of cryptococcal isolates with speciation and subtyping via molecular techniques.

8.2.4 Frequency of LPs

An initial LP is required for CM diagnosis at the time of enrollment with written informed consent. Therapeutic LPs will be performed at day ~3, day 7, day 10, day 14, and whenever clinically indicated for the reduction of elevated ICP as recommended by IDSA and DHHS guidelines.[63,70] Subjects with a positive CSF culture at day 14 (estimated ~35%) will be recommended to have a follow up LP by the week 4 outpatient visit to document whether ongoing culture positive cryptococcosis is continuing. A therapeutic LP conducted in the first week after cryptococcal diagnosis (i.e. as scheduled at day 3) reduces the relative risk of mortality by 69% in the first 10 days, regardless of initial ICP.[71]

The majority of individuals being treated for CM are expected to have sterile CSF cultures by week 4. Subjects with positive CSF fungal culture at week 4 will be recommended to have a follow up LP at 8-10 weeks to document clearance of the cryptococcal infection, prior to decreasing the fluconazole maintenance dosing. Until CSF is known to be culture negative, fluconazole will remain dosed at 800-1200mg. The following summarizes these recommendations:



Serial LPs are recommended as the standard of care for controlling elevated ICP in CM. The above diagram outlines a recommended sequence, though does not consider ICP. According to the current standard of care, if a subject has elevated intracranial pressure (>200 mm CSF), an LP should be performed therapeutically to reduce elevated intracranial pressure.[9,72]

All follow-up LPs must have verbal consent from the subject prior to performing the LP. As LPs are invasive procedures, subjects will need to give consent prior to performing follow up LPs. The initial diagnostic LP at screening visit requires *written* informed consent. Follow up LPs require *verbal* consent. Subjects may decline follow-up LPs at their own risk, but therapeutic LPs will continue to be offered, at minimum, on days 3 and 7.

Once subjects are CSF culture negative (given at least 7 days of culture growth) and have a normal CSF opening pressure (<200 mm CSF), they should not have a routine follow up LP, unless new symptoms develop. If new symptoms develop concerning CM-IRIS or CM relapse, an LP will be recommended to guide clinical management.

8.3 Laboratory Evaluations

All routine clinical tests as specified on the schedule of events will be performed at the local site laboratory.

8.3.1 Hematology

CBC monitoring will be encouraged as part of routine care at the start of CM therapy, initial evaluation for ART, and whenever clinically indicated. In prior analyses of 296 cryptococcal meningitis patients from Uganda enrolled in the COAT trial and ASTRO pilot study,[50] grade 3-4 anemia is very common

(42%) but has no association with 10-week mortality (19% with anemia vs. 20% without anemia). During the 2010-2013 COAT trial, the only death from anemia occurred in a person who received a blood transfusion and had transfusion-related acute lung injury (TRALI).[50] Thus, aggressive monitoring to detect anemia is likely unwarranted, if there is no positive benefit on improving survival.

8.3.2 Chemistry

Serum creatinine, potassium, and LFTs (AST, ALT, total-bilirubin) will be monitored at the local approved site laboratory. Serum aliquots of 0.5-1mL will be frozen at -80ºC.

Serum creatinine and potassium measurements should occur as per the schedule events so long as participants are receiving amphotericin. If participants end amphotericin before 14 days, then it is allowable to discontinue serum creatinine and potassium monitoring.

8.3.3 HIV Profile

CD4 T cell profiles will be encouraged on initial HIV diagnosis. HIV viral loads will be run selectively at medical officer discretion among those participants receiving ART at study entry with suspicion of virologic failure (i.e. receiving ART for >3 months) during the second week of hospitalization.

8.3.4 Stored Specimens

Any extra sera and plasma collected for clinical testing (e.g. creatinine and potassium) will be stored for later potential cytokine analysis (Bio-Rad, Hercules, CA) via a Luminex or ELISA instrument to investigate biomarkers, which predict risk of mortality and/or IRIS pathogenesis. Peripheral blood mononuclear cells (PMBCs) will be stored in Kampala. The volume of stored blood should always be <30mL for all pathogenesis studies.

The CSF supernatant will be frozen for pharmacokinetic, pathogenesis, and diagnostic studies. CSF cell pellets will be collected in Kampala for immunology studies.

If a subject later withdraws storage consent (after initial consent), their study chart should be marked for ‘No Storage,’ and the PIs notified in order to destroy any stored specimens.

8.4 Specimen Storage

Specimens processed at the study site will be per the laboratory protocols for the site lab for clinical tests. Extra serum, plasma, and CSF must be stored at -80^0 C.

8.5 Biohazard Containment

Appropriate blood and secretion precautions will be employed by all personnel during blood draws, lumbar punctures, and shipping and handling of all specimens for this study, as currently recommended by the CDC, the NIH, and national guidelines.

9 ASSESSMENT OF SAFETY

All new grade ≥4 events (not limited to a laboratory abnormality) will be documented as soon as the study team becomes aware. Safety is a secondary endpoint of the ASTRO-CM trial. AE severity will be graded using the NIH NIAID Division of AIDS AE Grading Table (version 2009).

ART will be administered through programs in accordance with the National ART Guidelines and supported by organizations such as the Ministry of Health, PEPFAR, and/or The Global Fund. The range of induction antifungal therapy being given (5-14 days) is within the recommended range of therapy per the WHO cryptococcal treatment guidelines.[73]

9.1 Reporting of Serious Adverse Events

Events requiring reporting to the regulatory authorities include:

a. All clinically serious adverse events irrespective of relationship to the health related intervention;
b. All serious *unexpected* events irrespective of relationship to health related intervention;
c. All clinical events associated with protocol violations irrespective of severity;

Reports will be submitted to the local IRB of record as per that IRBs specified reporting requirements. For serious unexpected events, these must be reported within 7 days of the study site awareness of the AE. Copies of each report and documentation of IRB notification and receipt (if provided) will be kept in the study site's Clinical Investigator's regulatory binder.

At the time of the initial report, the following information will be provided:

- Study Title
- Study Site
- Participant identification number
- Date of Report
- Date of Study Team's Awareness the Event
- A Description of the Event
- Working Diagnosis
- Name of the Reporting Investigator

Within the 7 days of the initial report (or concurrently), the investigator will provide further information on the "AE Reporting CRF" or study "Termination CRF" in the form of a written narrative. This will be documented along with any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing serious AEs will be provided promptly to the study sponsor and local IRB of record.

Reporting of AEs to other IRBs and regulatory agencies, which are not the local-IRB of record, will adhere to their requested reporting frequency, including the annual aggregate reports of the data from all study sites.

9.1.1 Local IRB of Record

The local IRB of record for study sites are:

- Mulago Research and Ethics Committee in Kampala, Uganda
- Mbarara University of Science and Technology IRB in Mbarara, Uganda
- Ifakara Health Institute IRB in Ifakara, Tanzania

9.2 Adverse Event (AE) Definitions

Adverse Event (AE)

An AE is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable or unintended sign (including abnormal laboratory findings), symptom, or disease temporally associated with the use of a medicinal product. Any symptoms, signs, illnesses or experiences that develop or worsen in severity during the course of the study are considered AEs.

Abnormal results of diagnostic or laboratory procedures are considered to be adverse events if the abnormality:

- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests, or
- is considered by the investigator to be of clinical significance

9.2.1 Serious Adverse Event

AEs are classified as serious or non-serious. A *Serious adverse event* is:

- fatal
- life-threatening
- requires re-hospitalization (after hospital discharge for the initial, pre-existing cryptococcosis)
- results in persistent or significant disability or incapacity
- results in congenital anomaly or birth defect
- an important medical event that may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above

All AEs that do not meet any of the criteria for serious will be classified as *non-serious adverse events*. Non-serious AEs do not require expedited reporting to the local IRB of record, regardless of the severity Grade.

9.2.2 Severity of Adverse Events

The term severity is defined as the intensity grade or level for a specific event, i.e., mild, moderate, severe, or potentially life-threatening. Importantly, severity is *not* the same as seriousness, which is based on participant/event *outcome or action* criteria usually associated with events that pose a threat to a subject's life or functioning (ICH E2A). The *Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (Clarification 2009)* will be used. For events not included in the protocol defined grading system, than the following guidelines will be used to quantify intensity:

Two severity grades for AEs are to be recorded:

Grade 4 = potentially life-threatening: any adverse drug experience that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e. it does not include a reaction that had it occurred in a more severe form, might have caused death.

Grade 5 = Death

9.2.3 Expected Adverse Events

Expected events include:

- Death due to cryptococcal meningitis (~40% of enrolled subjects);
- Symptoms due to CM, CM IRIS, or CM relapse;
- Transient renal insufficiency in the first 14 days after study entry due to amphotericin (~50%);
- Cryptococcal IRIS events (~20%); [50]
- Re-hospitalization or death due to IRIS, CM relapse, or persistently elevated ICP (20-25%);[9,10,15]
- Nosocomial infections, including bacteremia (~15%) [74]
- Aspiration pneumonia, due to altered mental status (~15%)
- AIDS-related opportunistic infections, including Tuberculosis;
- Common ART side effects and laboratory abnormalities (e.g. Grade 4 anemia ~33%); [50]

Due the nature of the participant population of persons with cryptococcal meningitis and AIDS, the expected adverse events (as above) will be frequent (>200% incidence of the enrolled participants) and unrelated to the study intervention. All open DSMB reports and findings will be submitted to the IRBs. The DSMB will have full access to unblinded outcome and AE data.

9.3 Adverse Event Collection Period

Adverse events will be collected from study entry through 18 weeks (or study termination).

9.4 Pre-existing Conditions

A pre-existing condition is one that is present at study entry. All persons have life threatening cryptococcosis. At enrollment, any clinically significant abnormality should be recorded on the 'Enrollment CRF' as a pre-existing condition. All pre-existing conditions must be clearly documented because new Grade ≥4 AEs are a study endpoint. If the frequency, intensity, or the character of the condition worsens to Grade ≥4 level during the 18-week reporting period, the event should be defined as an AE. At the end 18 weeks, any new clinically significant findings/abnormalities that meet the definition of an AE in retrospective comparison to the pre-existing conditions must also be recorded and documented as an AE(s).

9.5 Recording of Adverse Events

All Grade ≥4 AEs occurring during the study period will be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause (e.g. motor vehicle accident, illicit drug use). Serious AEs that are still ongoing at the end of the 18-week reporting period must be followed up to determine the final outcome. All unresolved AEs at the 18-week visit should be followed until the events are resolved or otherwise explained.

9.6 Safety Oversight

All IRBs will receive annual aggregate progress reports, including aggregate rates of AEs. These aggregate rates can be compared to the expected incidence of AEs (Section 9.2.3). The DSMB (Section 11.4) will perform interim reviews of unblinded data.

10 CLINICAL MONITORING

10.1 Site Monitoring Plan

Site monitoring will be conducted to ensure that the human subject protection, study procedures, laboratory, study intervention administration, and data collection processes are of high quality and meet the sponsor, ICH E6 and, when appropriate, regulatory guidelines. An internal quality monitoring plan will be in place with remote review of all digitally scanned source documents.

10.2 Laboratory Quality Control

All cryptococcal isolates will be stored in glycerol and frozen at ≤-20^{0}C for potential external verification at the University of Minnesota, Department of Microbiology laboratory of Dr. Kirsten Nielsen.

10.3 Depression Monitoring

Participants will be evaluated for depression using the Center for Epidemiologic Studies in Depression (CES-D) scale. If there is concern for severe depression, the participant may be switched to open label sertraline and/or referred to psychiatrist. Depression is known to occur in this population and improves with HIV therapy.

10.4 Safety Review

Subsequent review of serious, unexpected, and related AEs by the trial steering committee, IRB, the sponsors, or the relevant local regulatory authorities may result in suspension of further trial interventions. The study sponsors retain the authority to suspend additional enrollment and study interventions for the entire study, as applicable. Examples of findings that might trigger a safety review are the number of SAEs overall, the number of occurrences of a particular type of SAE, severe AEs/reactions, or increased frequency of events.

11 STATISTICAL CONSIDERATIONS

11.1 Study Hypotheses

We hypothesize that the addition of sertraline to standard CM induction therapy will result in improved survival. We will compare 18 week survival time between sertraline and placebo group as the primary endpoint.

11.2 Overview and Design Considerations

This is a phase III randomized trial to evaluate the impact of adjunctive sertraline on survival, when added to standard amphotericin-based therapy for CM. Diagnosis will be made via CSF cryptococcal antigen (CRAG) at time of lumbar puncture (LP) with confirmation by CSF culture. After informed consent, subjects with CM that meet eligibility requirements will be able to enter study. A non-randomized phase I dose-escalation study was conducted between Aug 2013 and Aug 2014 to help optimize dosing for the larger randomized phase III study. Details of the trial design are outlined in Section 4.

11.3 Statistical Power for the Primary Outcome

Table 8 summarizes the power associated with sample sizes necessary to detect a hazard ratio of 0.65, assuming a two-sided alpha = 0.05. With n=275 per group, we have 83% power when the proportion surviving in the control group is 60%, and 77% power when the proportion surviving is 65%. Given that we expect 60-65% survival over 18 weeks in the control arm, we have approximately 80% power with n= 275 per group, for a total sample size of 550 persons.

Table 8. Sample size and power to detect a hazard ratio for survival time based on:

	Event Rates		
Sample Size per group	Control Group	Treatment Group	Power
225	45%	32%	80%
	40%	28%	75%
	35%	24%	69%
250	45%	32%	84%
	40%	28%	79%
	35%	24%	73%
275	45%	32%	87%
	40%	28%	83%
	35%	24%	77%
300	45%	32%	90%
	40%	28%	86%
	35%	24%	81%

11.4 Statistical Power for Secondary Outcomes

Table 9 summarizes the sample sizes necessary for 80% and 90% power to detect differences (in terms of standard deviation) between the groups for continuous valued outcomes.

Table 9. Power table for continuous valued outcome. Table entries are the differences between the groups in terms of standard deviation (SD).

Sample Size per group	90% Power	80% Power
100	0.461 of SD	0.398 of SD
150	0.376 of SD	0.325 of SD
200	0.325 of SD	0.281 of SD

The CSF rate of *Cryptococcus* clearance, termed EFA, will be calculated for all enrolled participants with at least two LPs during study days 1-18. The EFA in the placebo arm is expected to be similar to the COAT study results: EFA mean $\pm$SD = -0.36 $\pm$0.20 $\log_{10}$ CFU/mL of CSF/day.[20] Assuming that standard deviation of $\pm$0.20 and 200 persons per group with an evaluable EFA, we have 90% power to detect a difference between the groups of 0.2*0.325 = 0.065, and 80% power to detect a difference between the groups of 0.056. Similar to phase II TB trials, the rate of CSF clearance has been used in CM trials to demonstrate efficacy.

To evaluate neurocognitive outcomes we also assume that the control group will have scores at 14 weeks similar to those in the COAT study at 3 months, where the mean $\pm$SD was -1.4 $\pm$1.1. Assuming a standard deviation of 1.1 and 150 persons per group with evaluations at 14 weeks, we have 90% power to detect a difference in QNPZ-8 scores between the groups of 0.414 and 80% power to detect a difference of 0.358.

Data Monitoring

Safety oversight will be under the direction of an independent Data and Safety Monitoring Board (DSMB) composed of five international experts in cryptococcosis, pharmacokinetics and biostatistics.

A quorum of 3 members (including the biostatistician) will be required for the DSMB to meet. The initial DSMB will be composed of:

- Mohammed Lamorde (IDI, Pharmacologist),
- Joseph Jarvis (Botswana-UPenn Partnership, Physician-Scientist),
- Mecky Matee (Muhimbili University, Tanzania, Microbiologist),
- Jason Baker (Hennepin County Medical Center,USA, Physician-Scientist), and
- Marcel Wolbers (Oxford University Clinical Trials Unit - Vietnam, Biostatistician).

A DSMB charter details the roles of the DSMB (Appendix B).

The safety and endpoint data will be reviewed after the first 25% of participants (~140) are enrolled, and will meet at least annually. DSMB members can also request additional more frequent reviews. The DSMB will recommend early termination of the study only when there is clear and substantial evidence of benefit or harm. For more details, refer to **Appendix B: Study Monitoring and Statistical Analysis Plan**.

11.5 Final Analysis Plan

All analyses will be intent-to-treat, with a two-sided alpha level of 0.05 with comparison of sertraline 400mg/day vs. placebo.

For the primary outcome, 18-week survival will be summarized by time-to-event analysis. Kaplan-Meier curves will be used to summarize the pattern of time-to-event over 18-weeks. Similar analyses will be performed for the secondary outcome of event free survival.

For the secondary outcome of assessing the early fungicidal activity (EFA) clearance, a mixed effects regression model with a random intercept for individual will be used to account for the intra-subject correlation induced by repeated measures over time. An interaction indicator variable of time and treatment groups will assess differences in clearance rates.

Subgroup analyses for the survival and EFA outcomes will be done according to the randomization strata (clinical site and ART use at baseline) and altered mental status at baseline (Glasgow Coma Score = 15 and <15).

Secondary endpoints that can be analyzed with time-to-event methods (time to sterility, IRIS, CM relapse, adverse events) will be summarized with cumulative incidence functions to account for the competing risk of mortality. Other categorical secondary endpoints will be summarized using logistic regression or Fisher's Exact methods as appropriate. Continuous-valued secondary endpoints will be compared with general linear models or Wilcoxon rank-sum tests as appropriate.

Baseline demographic features of each study arm will be summarized, with statistical testing as appropriate for nominal and continuous variables to assure adequacy of comparison.

A cost-benefit analysis will be performed considering the additional costs of receiving sertraline in addition to standard therapy in relation to: i) survival, ii) days of hospitalization, so as to understand whether this intervention (if effective) is cost-effective to implement.

Baseline characteristics known to be associated with mortality in CM (high opening pressure, lack of pleocytosis, normal CSF protein, high CSF CRAG titer, low CD4 count, altered mental status, and concomitant pulmonary disease) will be queried as to whether they are equally distributed among the two randomized arms. Any parameters found to be potentially different (P<0.1) may be included in a multivariable adjusted regression analysis.

12 SOURCE DOCUMENTS

The site will maintain appropriate medical and research records for this trial, in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects.

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, subjects' memory aid or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, digital or magnetic media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial. It is acceptable to use CRFs as source documents.

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 Ethical Standard

This study is to be conducted according to U.S. and international standards of GCP (International Conference on Harmonization guidelines), the Declaration of Helsinki, and International Ethical Guidelines for Biomedical Research Involving Human Subjects. It must also comply with applicable government regulations for Uganda, Tanzania, U.S., international and Institutional research policies and procedures. All investigators must have received human subjects' protection training prior to human subject involvement.

13.2 Institutional Review Board

Prior to the initiation of the study at the clinical research site, the protocol, all informed consent forms and the participant Information materials will be submitted to and approved by the local IRB of record. Likewise, any future amendments to the study protocol will be approved by each site's IRB. Please refer to Appendix A for informed consent documents.

This protocol and any amendments will undergo review and approval by the Human Subjects Board at the University of Minnesota, Mulago Research and Ethics Committee (MREC), Mbarara University of Science and Technology, the Uganda National Council of Science and Technology (UNCST), Ifakara Health Institute (IHI), and all other relevant local/national IRBs for any site.

Registration will occur with the Ugandan National Drug Authority (NDA), Tanzania Food and Drugs Authority (TFDA), and U.S. Food and Drug Administration (FDA).

13.3 Subject Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participating subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior

written approval of the sponsor. For research uses, all data will be de-identified and coded with the clinic medical record number.

Any authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

13.4 Study Discontinuation

In the event that the study is discontinued, subjects remain eligible for ART according to national guidelines. Participants will be encouraged to continue to receive ART from the outpatient clinic or referred to a clinic of their choice for ongoing HIV care and management of cryptococcosis.

13.5 Future Use of Stored Specimens

According to Ugandan regulations, a separate informed consent for long-term storage of samples for future research testing is necessary. After informed consent is obtained, the storage consent for storage of samples should be presented to consent subjects. Subjects have the option to consent for long-term storage of samples for this study only and/or for future research. If a subject declines storage consent, this will be noted on their lab order entry form and in their study chart, and specimens will be discarded after appropriate clinical testing is completed. The purpose of the long-term storage is to enable: 1) future diagnostic testing for assays which are not currently commercially available (e.g. TB-LAMP PCR, TB biomarkers); 2) pathogenesis studies to better understand immunology and microbiology of cryptococcal meningitis in relation to clinical outcome; 3) pharmacokinetic studies of antifungal medications.

Due to the preference of the Ifakara Health Institute IRB, long term storage will not occur in Tanzania, and specimens will be discarded at the final study conclusion.

14 DATA HANDLING AND RECORD KEEPING

The investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. Changes or corrections will be made by crossing out the original entry with a single line. All changes must be dated and initialed by the correcting party. The use of correction fluid or any other medium that may render the original entry illegible is not permitted.

Copies of the CRF will be provided for use as source documents and maintained for recording data for each subject enrolled in the study. Data reported in the CRF derived from source documents should be consistent with the source documents or the discrepancies should be explained.

14.1 Source Documents

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Study data forms will be digitally scanned for permanent record keeping and to enable rapid resolution of any discrepancies.

14.2 Data Management Responsibilities

All source documents and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Adverse events must be graded, assessed for severity and causality, and reviewed by the site PI or designee.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. During the study, the investigator must maintain complete and accurate documentation for the study.

14.3 Data Capture Methods

Data entry will occur via computer database using the DataFax system. The overall coordination will be the responsibility of the principal investigator.

14.4 Types of Data

Data for this study will include safety, laboratory (clinical, immunologic and microbiological), and outcome measures (e.g. survival).

14.5 Study Records Retention

The investigator will retain study essential source documents for 5 years after the completion of the study. Digital images of the source documents will be retained for an indefinite period. Specimens will be stored temporarily for the duration of the study, and will be discarded 1 year after final completion and publication of the study results. Long-term storage will not occur unless participants specifically consent to long-term storage.

14.6 Protocol Deviations

A protocol deviation is any noncompliance with the study protocol, Good Clinical Practice (GCP), or Manual of Operations (MOP) requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions will be developed and implemented promptly.

These practices are consistent with ICH E6:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site to use continuous vigilance to identify and report protocol deviations within 5 working days of identification, or within 5 working days of the scheduled protocol-required activity.

14.7 Quality Control and Quality Assurance

Each site will have a QC/QA Quality Management plan in place. The DataFax system incorporates quality assurance for complete record keeping.

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