1. TITLE PAGE

Short title: Cryptococcal Antigen Screening plus Sertraline (C-ASSERT)

Long Title: Randomized Clinical Trial evaluating sertraline plus fluconazole versus fluconazole alone for the preemptive treatment of asymptomatic cryptococcal antigenemia in HIV-infected persons living with AIDS

Clinical Trial Phase: II / III

Local Protocol Number(s):

UNCST Number: HS 2204

National Drug Authority: 584/NDA/DID/08/17

NIH NIAID Funding Mechanism: U01AI125003

Principal Investigator (PI): David Meya / David Boulware

IND Sponsor: *University of Minnesota*

FDA IND Number: 133772

Protocol Chair: Radha Rajasingham

NIAID DAIDS Medical Officer: Melanie Bacon

DAIDS-ES ID: 33238

ClinicalTrials.gov Identifier: NCT03002012

Version Number: 2.0

Version Date: 10th October 2017

SIGNATURE PAGE

I agree to conduct the study in accordance with the relevant, current protocol and will not deviate from the protocol without permission of DAIDS, except when necessary to protect the safety, rights, or welfare of study participants.

I agree to personally conduct or supervise this study.

I will ensure that the requirements relating to obtaining informed consent and Ethics Committee (EC) or Institutional Review Board (IRB) review and approval (45 CFR 46, ICH/GCP, etc.) are met.

I agree to report to the sponsor adverse experiences as per the protocol that occur during the course of this study.

I agree to maintain adequate and accurate study records and to make those records available for inspection by DAIDS, DAIDS' authorized representatives, and/or other applicable regulatory entities.

I will ensure that an EC or IRB that complies with the requirements of 45 CFR Part 46 will complete initial and continuing review and approval of the study. I also agree to promptly report to the EC/IRB: 1) all changes to the study; 2) safety updates DSMB summaries and other information required by the funder, sponsor and EC/IRB in compliance; 3) all unanticipated problems involving risks to human subjects or others. Additionally, I will not make any changes to the study without DAIDS and EC/IRB approval, except where necessary to eliminate apparent immediate hazards to study participants. All protocol changes should be reviewed and approved by the PI, DAIDs, and regulatory authorities.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Principal Investigator: _____

Signed:_____Date: _____

Name/Title

2. TABLE OF CONTENTS

2.1. Key Roles

David R Boulware MD, MPH, CTropMed. Dr. Boulware affiliated with the University of Minnesota is the U.S.-chair who will lead protocol development and senior advisor to the overall conduct of the project. He will work with the team to evaluate data in writing abstracts and manuscripts related to the data collected.

David B Meya MBChB MMed, PhD, Dr. Meya is the Ugandan-chair who led the protocol development, and will be responsible for supervision of study staff, overseeing study implementation, and ensuring that the implementation of the protocol is conducted with integrity based on NIH and local regulatory bodies.

Radha Rajasingham MD. Dr. Rajasingham is the U.S. protocol champion, who will develop the protocol, present the protocol to ethical bodies for review and approval. She will serve as an advisor for protocol implementation, and will work with the team to analyze data, write abstracts, and manuscripts related to the data collected.

Elizabeth Nalintya MBChB. Elizabeth is the study coordinator for the C-ASSERT trial, and will provide day-to-day supervision of the study staff and supervise project implementation in Uganda.

Conrad Muzoora MBChB, MMed is the site PI in Mbarara, Uganda who will provide input for protocol development and led protocol implementation in Mbarara, Uganda.

Freddie Mukasa Kibengo MMed is the site PI in Masaka, Uganda who will implement the trial in Masaka.

Melanie Bacon RH MPH, is the NIH NIAID DAIDS Medical Officer and representative of the funder.

Biostatistician:

Kathy Huppler Hullsiek PhD. Dr. Hullsiek is a biostatistician affiliated with the Division of Biostatistics at the University of Minnesota. She will provide statistical support during protocol development, and will be primarily responsible for data management, data analysis, and dissemination of results.

Additional medical officers will report to the Site PI of record.

The University of Minnesota is the regulatory sponsor of the C-ASSERT trial.

2.2. List of Abbreviations

AIDS	acquired immunodeficiency syndrome
ALT	alanine aminotransferase
ART	antiretroviral therapy
AUC	area under the curve
AZT	zidovudine
CBC	complete blood count
CFU	colony forming units
COAT	Cryptococcal Optimal ART Timing
CrAg	cryptococcal antigen
CRP	C-reactive protein
CSF	cerebrospinal fluid
DAIDS	Division of AIDS (NIH, NIAID)
DSMB	Data and Safety Monitoring Board
EKG	electrocardiogram
EFA	early fungicidal activity
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GCS	Glasgow Coma Scale
Hgb	Hemoglobin
HIV	human immunodeficiency virus
IDI	Infectious Disease Institute
IDSA	Infectious Diseases Society of America
IND	Investigational New Drug
IRB	Institutional Review Board
IRIS	immune reconstitution inflammatory syndrome
IQR	interquartile range
IV	intravenous
JCRC	Joint Clinical Research Centre
LFA	lateral flow assay
LP	lumbar puncture
MOP	manual of operations
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OI	opportunistic infection
OHRP	Office of Human Research Protections
PEPFAR	President's Emergency Plan for AIDS Relief
PO	*per os* (by mouth)
SOC	Standard of Care
SOPs	Standard Operating Procedures
QTc	corrected QT interval
UK MRC	United Kingdom Medical Research Council
UNCST	Ugandan National Council for Science & Technology

3. PROTOCOL SUMMARY

Short Title	Cryptococcal Antigen Screening plus Sertraline (C-ASSERT)
U01 Award Title	Operational Research for Cryptococcal Antigen Screening (ORCAS) of HIV Patients
Study Abbreviation	C-ASSERT
Intervention	Asymptomatic CrAg+ persons will be randomized to fluconazole (current standard of care) vs. fluconazole plus sertraline
Study Hypothesis	Cryptococcal-free survival will be improved by an enhanced package of CrAg+ preemptive care with the addition of adjunctive sertraline
Sample Size	600
Study Population	HIV-infected persons with cryptococcal antigenemia in blood without symptomatic meningitis, predominantly with CD4<100
Study Design	Randomized parallel design, double blind trial
Study Phase	Integrated Phase II / III
Participant Duration	6 months
Standard of Care (per WHO guidelines)	Fluconazole 800mg/day x 2 weeks, 400mg/day for 10 weeks, then 200mg/day through 6-months
Expected Mortality	The expected mortality is 25-30% with standard of care therapy; untreated 100% mortality occurs within 6 months
Intervention	Sertraline vs. Placebo for 16 weeks Escalating doses up to 400mg/day Week1 : 100mg/day Week2: 200mg/day Week3: 300mg/day Weeks 4-12: 400 mg/day Weeks 13-15: Taper 300, 200, then 100mg/day
Rationale for Sertraline Dose	• Efavirenz induces hepatic P450 metabolism of sertraline • Usual 200mg antidepressant sertraline dose has ~50% lower levels in Ugandans receiving HIV therapy • Higher 400mg/day dose is needed to compensate for this metabolism, otherwise sub-therapeutic levels will occur. • At 400mg, 95% of participants are projected to achieve therapeutic concentrations of sertraline in brain tissue based on Ugandan pharmacokinetic data (n=140) and *Cryptococcus* susceptibilities of $\leq$4 μg/mL for sertraline.

Primary Objective	To determine if adjunctive sertraline provides a cryptococcal-free survival benefit in persons with asymptomatic cryptococcal antigenemia who have a 25-30% risk of death with current standard of care.
Secondary Objective	To identify incidence and risk factors for meningitis or death in asymptomatic CrAg+ persons To evaluate safety of sertraline in 300 HIV-infected persons
Primary Endpoint	6 month meningitis-free survival
Secondary Endpoints	• 6-month Survival Time • Incidence of Symptomatic Cryptococcal Meningitis • Incidence of Clinical Grade 3,4,5, and/or Serious Adverse Events • Incidence of Grade 3-5 Laboratory Adverse Events • Incidence of premature study drug/placebo discontinuation • Prevalence of depression by PHQ-9 score
Inclusion Criteria	1. HIV-infected 2. Cryptococcal antigen (CrAg) positive in blood 3. Age >=18 years and <65 years 4. Written informed consent 5. Women of childbearing potential who are participating in sexual activity that could lead to pregnancy must agree to use one reliable method of contraception while receiving fluconazole >=400mg/day
Exclusion Criteria	1. Prior history of cryptococcal meningitis 2. Suspected meningitis or mania 3. Suspected/known cirrhosis, jaundice, or ALT >5x ULN 4. Receiving an antidepressant medicine 5. Receiving antifungal therapy, >1 week 6. Breastfeeding / Pregnancy 7. Contraindication to Sertraline or fluconazole 8. Current rifampin use or other prohibited medication 9. EKG QTc >450ms
Safety Monitoring	1. In person visits will occur at baseline, 2 weeks, 4 weeks, and every 4 weeks thereafter. 2. Phone calls will be conducted for the first ~100 participants in the Phase II trial at weeks 1, 3, and 5 to assess wellbeing and any thoughts of self harm. 3. Participants will be provided contact numbers for study staff. If ill, transport will be provided to return to clinic/hospital

	for appropriate diagnostic evaluation with medical care provided to treat new adverse events. 3. Enhanced safety monitoring will occur with monitoring at baseline, 4, 8, and 12 weeks of: • Complete blood count • Serum Na^+, K^+, creatinine, ALT, total-bilirubin • EKG rhythm and QTc interval • Depression and suicidal ideation screening via PHQ-9 survey instrument Safety oversight is via the NIH NIAID HIV Complications & Co-infections data and safety monitoring board (DSMB). Sertraline is an FDA approved medication (since 1991) which is prescribed without any monitoring. Unlike other older antidepressants, there is a wide safety margin for SSRIs. In overdoses, consensus guidelines recommend no medical evaluation at sertraline doses <1250mg with at home observation (Protocol Section 8.1.8). Among 218 persons with cryptococcal meningitis who received sertraline 400mg/day for 2 weeks then 200mg for 8 weeks in the 2015-2017 ASTRO-CM trial, no participant had a sertraline-related grade 3-5 or serious adverse event.
Phase II Trial Activities for enhanced safety oversight	• Contact with participants will occur weekly for the first 5 weeks after randomization. Clinical symptoms will be assessed as will any concerns for self-harm (suicide). • A Trial Safety Committee will review all unexpected adverse events as they occur or any potential sertraline-related adverse events (Section 12.7.4). • Early Safety Independent Reviews will occur after every 40 subjects with a trigger for action of either: o >50% aggregate mortality + SAE incidence which is higher than ~40% expectation. (Section 12.7.5.) o ≥5% incidence of sertraline-related adverse event (i.e. 1 out of each 40 randomized = 1 in 20 on sertraline) • Safety reviews will continue every 40 participants until DSMB assumes responsibility for monitoring safety. • The Trial Safety Committee will review these safety reports and shall be empowered to: 1) Pause further enrollment; 2) Request early DSMB review; and/or 3) Request further interim safety reviews.

FDA Data on SSRIs and risk of suicide	The U.S. FDA mandates a black box warning on all selective serotonin reuptake inhibitors (SSRIs) that use in adolescents and adults <25 years old with depression may increase the risk of suicidality. "Suicidality" is defined as: thoughts of self harm, suicide preparation, suicide attempts, or suicides. This is based on a FDA safety report on 16 Nov 2006 which analyzed 372 anti-depression trials with 99,839 subjects. Any antidepressant medicine was associated with 2.3 fold higher odds of suicidality in adults <25 years, with SSRIs have a statistically significant result for the drug class. When sertraline was consider individually, the risk of suicidality was four-fold statistically lower (Odds Ratio = 0.25, 95%CI, 0.29 to 0.90, *P*=0.03) for subjects given sertraline relative to placebo for suicide preparation, attempts, or suicide among adults with psychiatric disorders. In young adults <25 years old, sertraline had non-statistically lower Odds Ratio of 0.61 (95%CI, 0.15 to 253, P=0.50). Among 57 sertraline trials, there were no suicides in the 2126 subjects assigned to sertraline but three suicides among 1733 placebo subjects and six suicides among the 2143 subjects assigned to other antidepressant drugs. FDA report is at www.fda.gov/ohrms/dockets/ac/06/briefing/2006-4272b1-01-FDA.pdf
Combined Trial activities	Phase II activities for the first ~100 participants are informative for trial continuation. If unexpected adverse events occur with sertraline use, the trial may be halted by trial safety committee or DSMB, but such events are unlikely to be statistically significant based on a ~100 participant sample size. All participants (Phase II and Phase III) will contribute data for the overall primary event. The overall trial has adequate sample size and statistical power to detect clinically relevant differences in trial endpoints.

3.1. Participating Sites

1) Infectious Diseases Institute (IDI) / Mulago Hill complex, Kampala, Uganda.
2) Joint Clinic Research Center (JCRC) – Mbarara Regional Centre of Excellence
3) Medical Research Council (MRC) / Uganda Virus Research Institute (UVRI) Masaka Field Station

A current list of study sites and the site lead investigator and/or points of contact at those sites is contained in study Appendix C. Minor additions, deletions, or substitutions from this list may occur over time and hence this list is potentially subject to revision. Appendix C will be updated with the most current list annually at the time of the Continuing Review

(if there are any updates), rather than submitting any interim updates in the form of formal protocol amendments. DAIDS approval will occur prior to any sites participating.

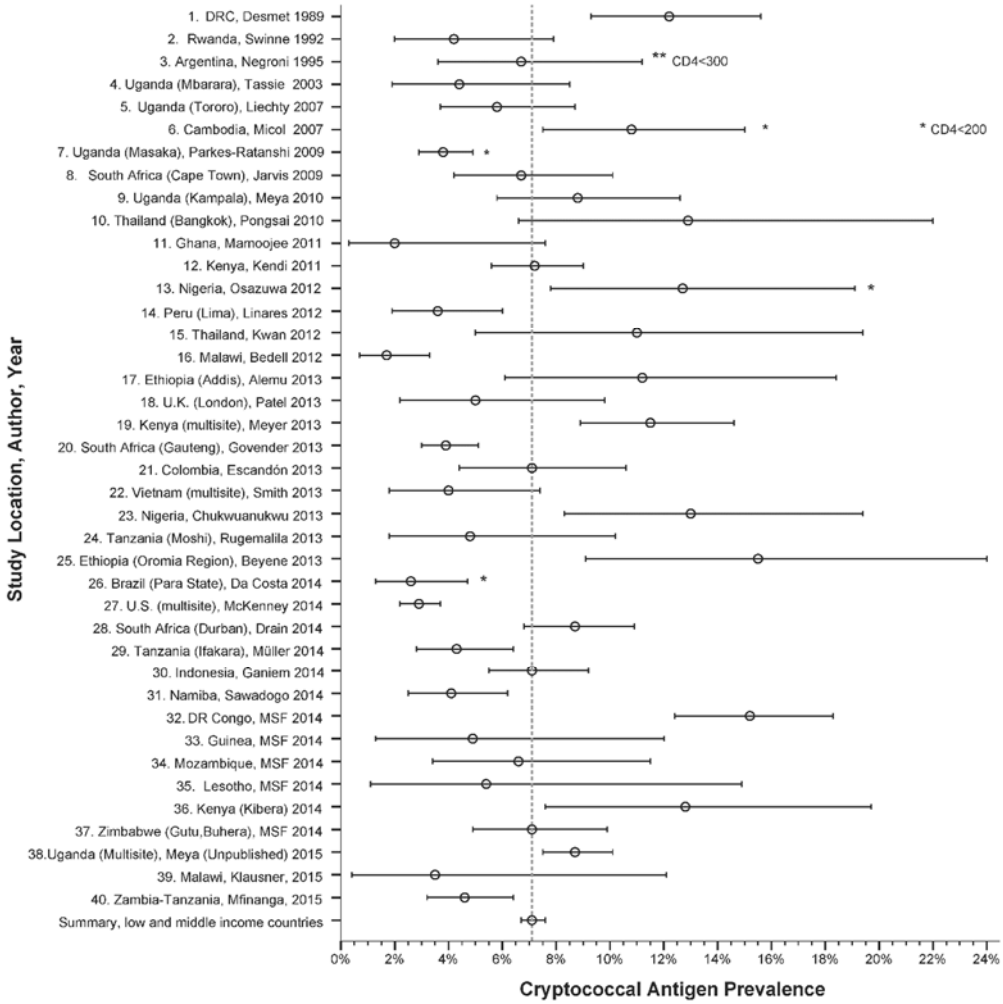
4. INTRODUCTION

4.1. Background on Cryptococcal Antigen Screening

In resource-limited settings, despite major efforts to expand ART access, the median CD4 T cell counts of patients initiating ART remain low with 20-25% of persons presenting with CD4<100 cells/μL. Cryptococcal disease is distinct from many other infectious diseases in that **asymptomatic persons with subclinical disease can be detected** 3 weeks to >3 months prior to the development of clinical, symptomatic cryptococcal meningitis.[1, 2] Asymptomatic persons living with AIDS and cryptococcal antigen (CrAg) in blood are common throughout Africa (**Figure 1**) averaging 7.2% (95% CI, 6.8-7.6%) in 38 studies of 14,815 persons from low and middle income countries **(Figure 1).**[1, 3-10] This is not a historical legacy, as 21 of 38 CrAg prevalence studies have been reported in 2013-14.

Cryptococcal antigen (CrAg) is a strong predictor of development of cryptococcal meningitis. ART alone is insufficient for asymptomatic CrAg+ patients with CD4<100; both antifungal therapy and ART are needed to reduce mortality.

Figure 1. Cryptococcal Antigen Prevalence (±95%CI)



A potential public health strategy to reduce AIDS-related mortality is to screen at-risk persons, using CrAg testing of serum or plasma, followed by targeted pre-emptive antifungal therapy for those testing positive.[3, 4, 11] Recent work from South Africa reported that a *positive* serum or plasma CrAg ≤2 weeks prior to ART initiation was 100% sensitive for detecting the development of meningitis in the first year of ART.[4]

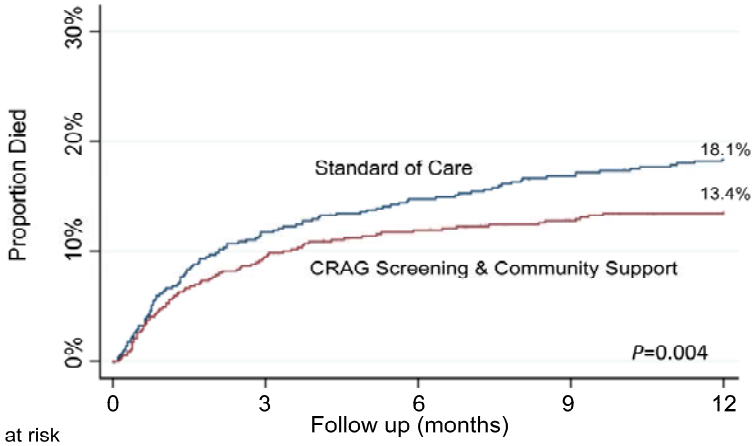
CrAg+ patients have substantial risk of cryptococcal disease and mortality despite ART.[1, 3, 4] Drs. Meya and Boulware (co-PIs) reported from a Ugandan cohort that all CrAg+ persons with a CD4 <100 cells/μL at time of ART initiation who received ART but not anti-fungal therapy died within 3 months of ART initiation (median CD4 = 30 cells/μL).[3] This 100% mortality without fluconazole was also found in Ifakara, Tanzania.[12] Conversely, there is 100% negative predictive value for CrAg-negative persons who receive ART to not develop cryptococcal meningitis.

Pre-ART CrAg screening is recommended by WHO and PEPFAR.[13, 14]

Our team has reported CrAg screening to be a highly cost-effective intervention in Africa, using the WHO Commission on Macroeconomics and Health criteria, costing only **$21** per disability-adjusted life year (DALY) saved (95%CI: $15-$32), based on a 8.8% CrAg+ prevalence and 3-year outcomes in Uganda but not including avoided hospitalization costs.[3] This estimate used the high cost CrAg latex agglutination test ($16) versus the new $2 CrAg lateral flow assay (LFA). Based on South Africa cost data, Dr. Joseph Jarvis (Advisory Committee) *et al* estimated that **CrAg screening saves $156 (95%CI: $119 to $197) for every person with a CD4<100/μL**,[15] and preemptive CrAg+ therapy **results in 40-80% better 5-year survival**.[3, 16] Jarvis *et al*'s cost savings estimate is not just among those CrAg+, but every person with CD4<100/μL, resulting in major cost savings to the health system.

CrAg screening with fluconazole preemptive therapy has proven to be efficacious in one published randomized clinical trial in Zambia and Tanzania (**Figure 2**).[17] In this trial, a **28%** relative reduction in mortality with CrAg screening and ART adherence support occurred overall in persons with CD4<200 cells/μL.

Figure 2. CrAg screening improves survival



Number at risk

CRAG screening:	1001	899	869	854	842
Standard of care:	998	869	834	811	794

4.2. Problem

Yet, the optimal management of those with asymptomatic cryptococcal antigenemia is unclear. ART alone is NOT sufficient to prevent clinical cryptococcal disease or death.[3, 4, 18, 19] In Kampala, our group prospectively demonstrated that preemptive fluconazole therapy is effective at improving short term and long term survival when starting ART.[3] However, in this observational, small, prospective cohort (n=33 CrAg+), fluconazole preemptive therapy was not standardized. Varying doses and durations were used between 200-400 mg for 2-4 weeks.[3] Of those CrAg+ with CD4 <100 cells/μL who received fluconazole, 15% developed meningitis and 29% died during follow up. [3] Longley *et al* reported higher fluconazole doses of 800-1200 mg have greater fungicidal activity than 400mg.[20] In 2011, the WHO guidelines for cryptococcal disease recommended CrAg screening in those with a CD4 <100, followed by preemptive therapy for those who were asymptomatic CrAg+.

WHO recommended preemptive therapy: 800mg fluconazole daily for 2 weeks, followed by 400mg daily for 8 weeks, "and continued maintenance with fluconazole 200 mg/day is recommended. The optimal antifungal regimen in this population remains to be determined."[13, 21] This is now standard of care per Ugandan national HIV guidelines, and is standard of care.

Notably, this is a conditional recommendation based on low quality evidence.[22]

Six-month mortality for those asymptomatic CrAg+ persons treated with this regimen, however, has remained high at 32% (**Figure 3**). We seek to provide better evidence on the treatment and risk factors for asymptomatic CrAg+ populations.

4.2.1. Standard of care CrAg screening in Uganda

Per Ugandan National HIV Guidelines, all ART-naïve persons with HIV-infection and a CD4<100 cells/μL should be screened for CrAg in the serum or plasma, irrespective of symptoms. Preemptive antifungal therapy is recommended for those asymptomatic CrAg+. The dosing of fluconazole is outlined above.

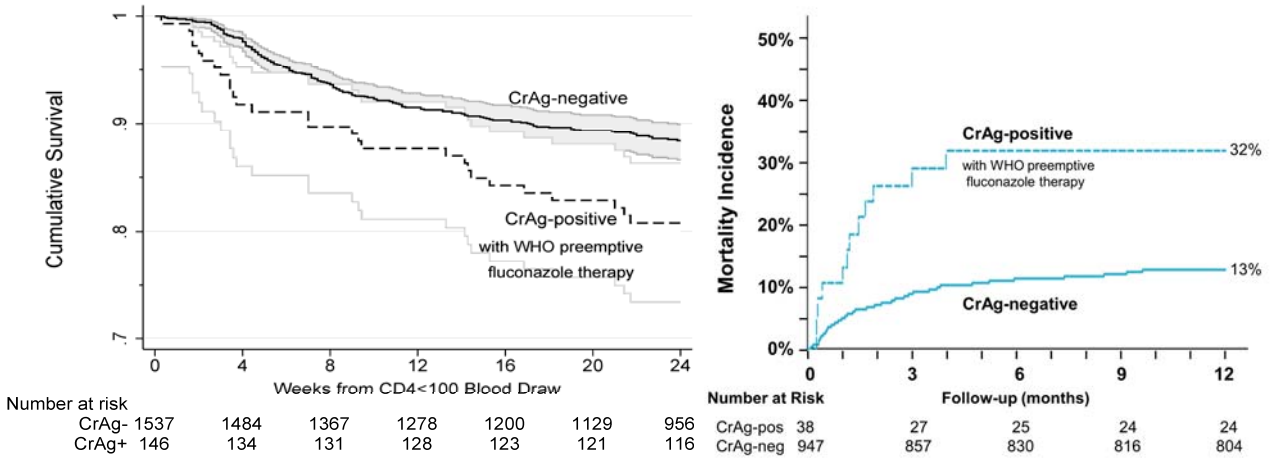
Ugandan national guidelines do not currently include secondary fluconazole prophylaxis for asymptomatic CrAg+ persons. WHO guidelines are equivocal regarding secondary prophylaxis. South African guidelines recommend 200mg of fluconazole daily for 6 months for those with asymptomatic cryptococcal antigenemia, after completing the above WHO recommended regimen. The benefit of this lengthened therapy is not known. To be released, 2016 Ugandan HIV guidelines will recommend fluconazole 200mg/day secondary prophylaxis through 6 months. In this trial we will follow Ugandan national guidelines. Thus, we will follow the above regimen, however if guidelines are changed during the course of this trial, we will follow the updated recommended regimen.

4.2.2. Evidence gaps in management of asymptomatic CrAg+

a) **Optimal preemptive antifungal therapy** is unknown

Although CrAg+ persons receiving fluconazole generally do better than if not treated at all, 25-30% go on to develop meningitis or die despite the WHO recommended antifungal therapy and ART. A 2.9-fold worse survival was observed in asymptomatic CrAg+ vs. CrAg- persons with CD4<200 in Tanzania and Zambia (**Figure 3** right).[17]

Figure 3. Survival by CrAg status in Uganda & REMSTART Trial in Tanzania & Zambia



One of the major, unresolved problems with CrAg screening is that CrAg+ individuals still have 2 to 3-fold higher mortality, despite receiving WHO recommended preemptive

fluconazole therapy (**Figure 3**). Despite preemptive therapy, CrAg+ individuals accounted for 12% of deaths. As cryptococcosis accounts for ~15% of HIV-related mortality,[1, 2, 23-26] this is a significant problem.

b) **Potential additional therapies** include sertraline and amphotericin. Flucytosine is another antifungal of interest, but flucytosine is not currently available in most low and middle income countries and is cost prohibitive in the U.S. ($2000/day).[27]
Timing of ART in asymptomatic CrAg+ has not been studied

In general, earlier initiation of ART results in earlier viral suppression, reduced risk of HIV transmission, and enhanced clinical outcomes. In other opportunistic infections, including TB, ART is also initiated as soon as possible. However, in cryptococcal meningitis a randomized control trial demonstrated excess mortality associated with early ART, likely due to immune reconstitution inflammatory syndrome (IRIS). Given this exception to the rule, an interesting area of future study is the timing of ART in asymptomatic CrAg+ persons. For this study, participants will be recommended to initiate ART at approximately 2-4 weeks after entry into care in line with Ugandan national guidelines.

c) **Risk factors for progression** to fulminant meningitis

Asymptomatic CrAg+ are at increased risk of meningitis and death. But who progresses to meningitis and the expected time frame of this progression has not previously been studied.

Lumbar punctures have been proposed as a valuable tool to investigate potential CNS involvement. However, lumbar punctures are generally very poorly accepted in African populations, and understandably, in a notably *asymptomatic* population. Furthermore, the majority of clinics do not have the capacity or healthcare worker resources to perform lumbar punctures on a number of asymptomatic outpatients. Thus this is not currently part of the Ugandan guidelines. One group in Tanzania performed lumbar punctures in 31/32 CrAg+ persons, and identified 2 out of 12 patients without any neurologic symptoms who had meningitis in CSF. Thus, the majority of persons with meningitis do have symptoms.

CrAg titers have been found to predict risk of meningitis and death. Other risk factors have not yet been evaluated.

d) **Need for secondary prophylaxis**

After initial therapy with fluconazole 800mg daily x 2 weeks, then 400mg daily x 8 weeks, it is unknown if there is additional benefit of giving patients 200mg daily of fluconazole thereafter. After fulminant meningitis, patients are given secondary prophylaxis with 200mg/day.

WHO, South Africa, US, Uganda, and other guidelines differ. Specifically, the

WHO 2010 Rapid Advice conflicts itself. The 2016 WHO guidelines and revised 'to be released' 2016 Ugandan guidelines recommend secondary fluconazole prophylaxis, based on no data, but some ongoing mortality after 10 weeks.

The large public health importance is that if secondary prophylaxis is necessary, then the cost of fluconazole preemptive therapy for CrAg+ increases by ~40%. We hypothesize based on timing of mortality (Figure 3) that secondary prophylaxis has minimal benefit when giving ART.[3, 17]

4.3. Study Hypothesis

We *hypothesize* cryptococcal-free survival will be improved by an enhanced package of CrAg+ preemptive care with the addition of adjunctive sertraline.

4.4. Rationale: Antifungal Effect of Sertraline

Sertraline. Sertraline, the selective serotonin reuptake inhibitor (SSRI) anti-depressant, has *in vitro* and *in vivo* activity against *Cryptococcus neoformans*.[28, 29] Sertraline's **mechanism of action** is interference with *Cryptococcus* protein synthesis via eukaryotic translational initiation factor 3 (Tif3).[29]

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.[30] Subsequent *in vitro* studies demonstrated that sertraline was fungicidal against both *Candida* and *Aspergillus* species,[30, 31] though at minimum inhibitory concentrations (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*.[29] When *in vitro* susceptibility testing was extended to pathogenic yeasts, particularly potent antifungal activity was observed against *Cryptococcus*. 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.[29] The methods and specific findings are summarized in Table 1.

Table 1: Major Findings of Sertraline's Anti-Cryptococcal Properties

Major Conclusions	Methods	Specific Findings [29]
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	• Comparison of sertraline and fluconazole susceptibilities • Time course assay comparing monotherapy vs combo therapy	• Combination therapy accelerated clearance • Larger and clearer halo in disks containing combo therapy

when combined with fluconazole	• Quantification of additive effects by calculating FFCI	• 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 1**), sertraline inhibited *C. neoformans* with an MIC between 2-6 µg/mL.[29] In addition, sertraline appeared to be fungicidal since killing was independent of cell proliferation (**Figure 4A**). 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 4B**). 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 *Cryptococcus*. In whole-genome deletion screening of *Saccharomyces cerevisiae* for instance, genes related to protein synthesis were highly enriched in sertraline-resistant strains (**Figure 4C**).

Figure 4. Antifungal *in vitro* and *in vivo* Activity of Sertraline [29]

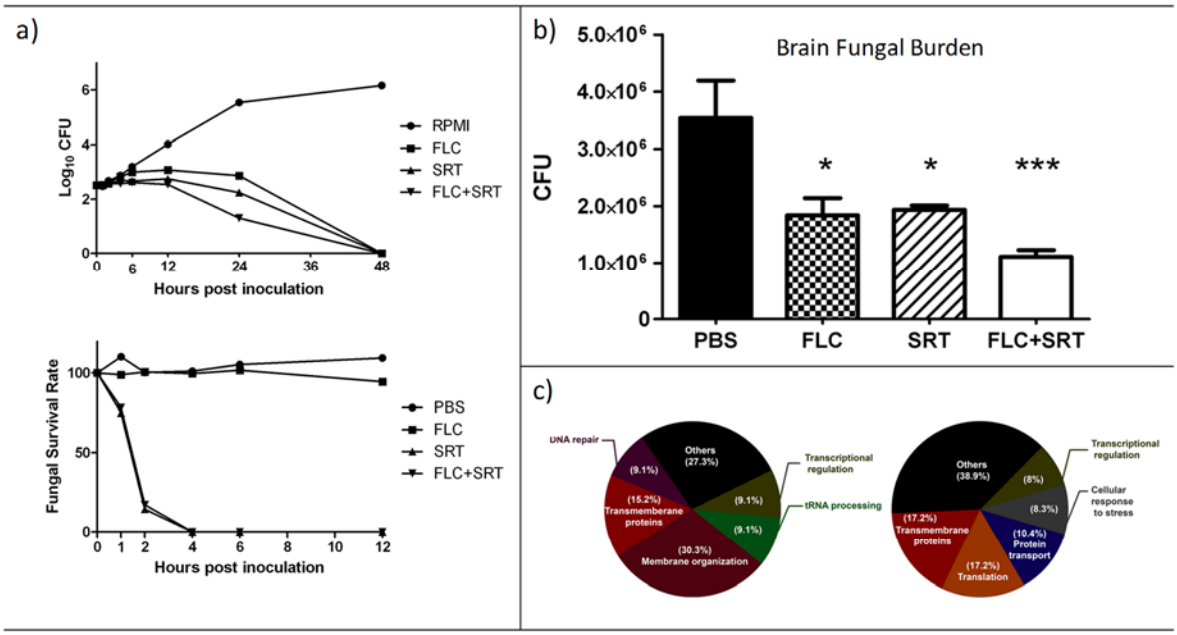


Figure 1. Antifungal properties of sertraline. **(a)** Sertraline is fungicidal against both proliferative and quiescent Cryptococcus. Cells were inoculated into media and cultured without any drug (control), or in the presence of fluconazole (FLC, 8 µg/ml), sertraline (SRT, 10µg/ml), or a combination 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).

4.5. Validation: Antifungal Effect of Sertraline

Our research group has validated the *in vitro* sertraline activity among 198 clinical *Cryptococcus* isolates from Uganda collected during the COAT trial (2010-2012),[32] 128 clinical isolates from Uganda during 2013-2014[33] and 153 clinical isolates from Mexico (Trevino-Rangel).[34] Additionally, Trevino-Rangel *et al* also completed a murine model of cryptococcal infection, replicating the findings of Zhai *et al*. [29, 34]

Table 2. *Cryptococcus in vitro* Susceptibility to Sertraline

Isolate Location	N	Concentration MIC (µg/mL)							
		1	2	3	4	5	6	8	12
Global [29]	24		2 (8.3%)	4 (25%)	10 (67%)	5 (88%)	3 (100%)		
Uganda 2010-12 [32]	198	19 (10%)	35 (27%)		95 (75%)		16 (83%)	32 (99%)	1 (100%)
Mexico [34]	153	62 (40.5%)	62 (81%)		26 (97.4%)			3 (100%)	
Uganda 2013-14 [33]	128	10 (7.8%)	25 (27%)		73 (84.4%)		9 (91.4%)	11 (100%)	
Pooled Results	**503**	**91 (18%)**	**124 (43%)**	**4 (44%)**	**204 (84%)**	**5 (85%)**	**28 (91%)**	**46 (99.8%)**	**1 (100%)**

Numbers represent N (cumulative percent).

The therapeutic target concentration in brain is 4 µg/mL for ~84% of *Cryptococcus*.

In our open label ASTRO trial for cryptococcal meningitis, when sertraline was added to amphotericin + fluconazole 800mg/day, the **rate of CSF yeast clearance was 23% faster with adjunctive sertraline** than historical cohort of the COAT trial who received amphotericin and fluconazole 800mg/day (**Table 3**, *P*=0.05).[35, 36]

Table 3: Early Fungicidal Activity (EFA) of the CSF *Cryptococcus* Clearance Rate by Regimen

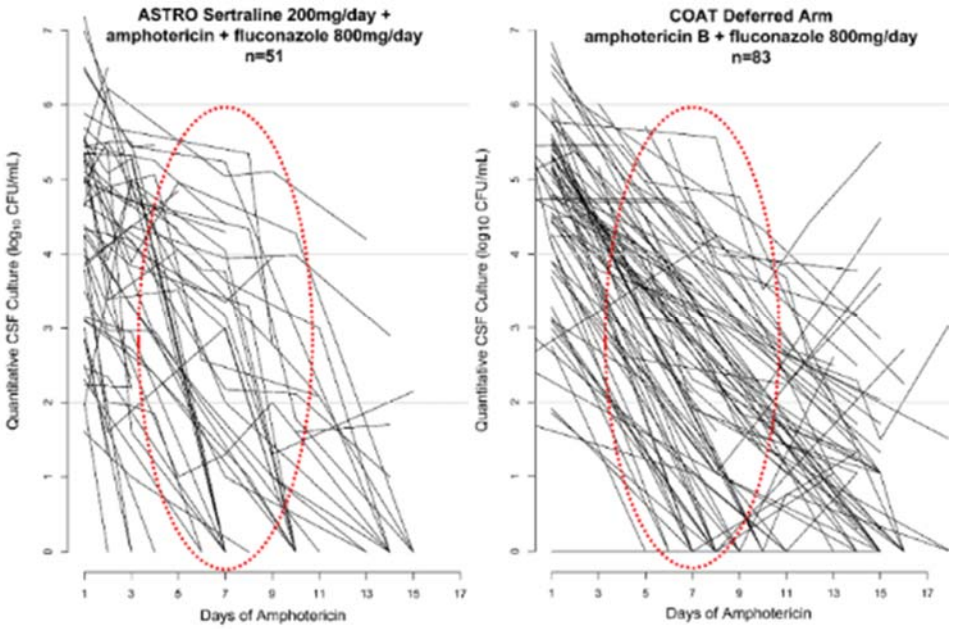
Trial	Treatment Regimen	N	**-EFA Mean** (95% CI) log_{10} CFU/ml/day
COAT	Amphotericin B 0.7-1 mg/kg/day + Fluconazole 800 mg/day	189	0.30 (0.28 to 0.32)
ASTRO Pilot	Ampho B + Flu800 + sertraline 100-400mg/day, pooled doses	128	0.37 (0.33 to 0.42)

EFA values are population means estimated with longitudinal mixed models with 95% CI

Skepticism regarding sertraline as an antifungal.

As displayed in **Table 3**, sertraline when added to standard antifungal therapy resulted in a 23% faster rate of CSF clearance. **Figure 5** displays the early fungicidal activity (EFA) plots of this increased fungicidal activity. The EFA plots display the quantitative CSF culture data (log_{10} CFU/mL) vs. days of induction therapy. Somewhat noteworthy is comparing days 5-9 when CFU counts plummet with adjunctive sertraline, near when sertraline is reaching steady state levels (Day ~7). The comparison is the COAT trial which received the same background antifungal regimen of amphotericin B + fluconazole 800mg/day, where clearance is linear.[35]

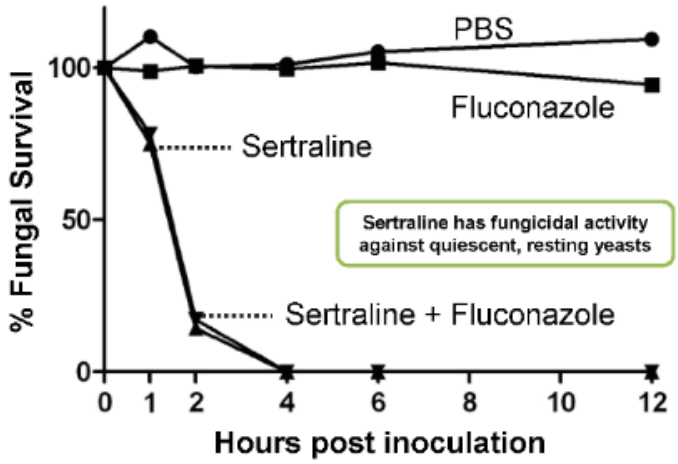
Figure 5. CSF *Cryptococcus* Clearance Rate



Unlike fluconazole, **sertraline has fungicidal *in vitro* activity against quiescent yeasts,** which are not proliferating (**Figure 6**).[29] This may have an important clinical effect.

In our open label ASTRO study (n=172), sertraline at ≥200mg/day eliminated cryptococcal relapse (0%) and greatly decreased paradoxical IRIS (<5% suspected IRIS) in comparison with COAT trial experience of 5% relapse and 17% paradoxical IRIS incidence.[35] We hypothesize sertraline's activity against quiescent yeasts in brain tissue and/or fluconazole synergy resulted in reduced relapse. This effect may be very important in subclinical infection in CrAg+ persons to eliminate the reservoir of quiescent yeasts.

Figure 6. *Cryptococcus* Survival in minimal media



In summary, the data supporting antifungal activity of sertraline include: 1) *in vitro* activity on clinical isolates; 2) *in vivo* murine model; 3) mechanism of action (post-translation protein inhibition); 4) human data in persons with cryptococcal meningitis on increased rate of CSF clearance, less relapse, and less paradoxical IRIS.

Sertraline is a generic medication with ≥25 manufacturers worldwide, >30 million U.S. annual prescriptions (with an excellent safety record), and the U.S. wholesale cost of 300mg/day is $2.15 per week. This is less expensive than flucytosine (5FC) ($4350/week), and unlike 5FC, sertraline is available in pharmacies in Africa. Efavirenz decreases sertraline levels by 25-50%; however, there is no sertraline effect on antiretroviral medicines.[37, 38]

4.6 Experience in Higher Dose Sertraline

A variety of early trials and studies investigated higher dose sertraline. In general, higher dose sertraline was well tolerated but did not have increased anti-depressant effects.

1) The ASTRO pilot study enrolled 172 HIV-infected participants with cryptococcal meningitis of whom 95 participants received sertraline starting at 300-400 mg/day. AEs were common in this population, but there incidence of AEs did not differ compared to sertraline at 100-200 mg/day.[33]
2) The ASTRO-CM randomized trial has enrolled 320 HIV-infected Ugandan participants with symptomatic cryptococcal meningitis (~50% randomized to sertraline 400mg/day) and 43 with cryptococcal relapse consented to open-label sertraline for compassionate care as of 19 October 2016. Overall the incidence of adverse events with a background therapy of amphotericin B deoxycholate and fluconazole 800mg/day is within expectations, being similar and slightly less than the prior COAT trial (**Appendix D**).[35] No adverse events have been attributed to

study medicine. **Tolerability has been good, as only three participants (0.8%) have discontinued the ASTRO-CM study medicine** (blinded sertraline/placebo).

3) In a trial of 66 participants with obsessive-compulsive disorder, high dose 250-400mg/day (mean 357mg) for 16 weeks of 36 persons had similar safety profile as 200mg/day.[39] Two persons discontinued therapy due to adverse events at the 200mg/day dose and none at the 400mg/day dose.[39] At high dose, the most common adverse events were insomnia (20%), diarrhea (16.7%), nausea (13.3%), and somnolence (13.3%).[39] These are similar (and lower) than listed in the package insert.[40]

4) EEG studies testing 400mg showed dose-dependent change in CNS activation and a widening of the pupillary size.[41]

5) Current Pfizer trial investigating sertraline at 400mg/day for lack of effect of sertraline on EKG QTc (n=54) has been completed as of 11 Oct 2016 (ClinicalTrials.gov: NCT02651623) without data available.

6) There is substantial experience in over-dose attempts using SSRIs.[42-44] Among 48,000 U.S. Poison Center calls regarding SSRI overdoses/exposures in 2004, consensus evidence based guidelines recommend for acute SSRI ingestions of sertraline of up 1250mg for observation at home among asymptomatic patients or those with mild effects.[44] Among an Australian SSRI overdose cohort, among 103 sertraline overdoses (median 4800mg, IQR, 3200-6000mg) ingestion, non-severe serotonin syndrome occurred in 20% and the EKG QTc was 6ms longer than a comparison control cohort (429 vs. 423ms) with 6% (6/103) of sertraline overdoses having a QTc >500ms.[43]

5. OBJECTIVES

5.1. Primary Objective(s)

- To determine if adjunctive sertraline improves 6-month cryptococcal meningitis free survival with retention-in-care over fluconazole preemptive monotherapy, which has a 25-30% risk of death with current standard of care.

5.2. Secondary Objective(s)

- To evaluate the safety of sertraline in asymptomatic CrAg+ persons.
- To identify incidence and risk factors for meningitis or death in asymptomatic CrAg+ persons.

5.3 Study Hypothesis

We *hypothesize* cryptococcal-free survival will be improved by an enhanced package of CrAg+ preemptive care with the addition of adjunctive sertraline.

5.4 Primary Endpoint

1) 6-month cryptococcal meningitis free survival with retention-in-care

5.5 Secondary Endpoints

1) 6-month survival time
2) Incidence of symptomatic cryptococcal meningoencephalitis
3) Incidence of Clinical Adverse Events (Grade 3-5)
4) Incidence of Grade 3-5 adverse laboratory abnormalities
5) Incidence of all-cause premature study drug discontinuation
6) Prevalence of depression by Patient Health Questionnaire (PHQ-9)

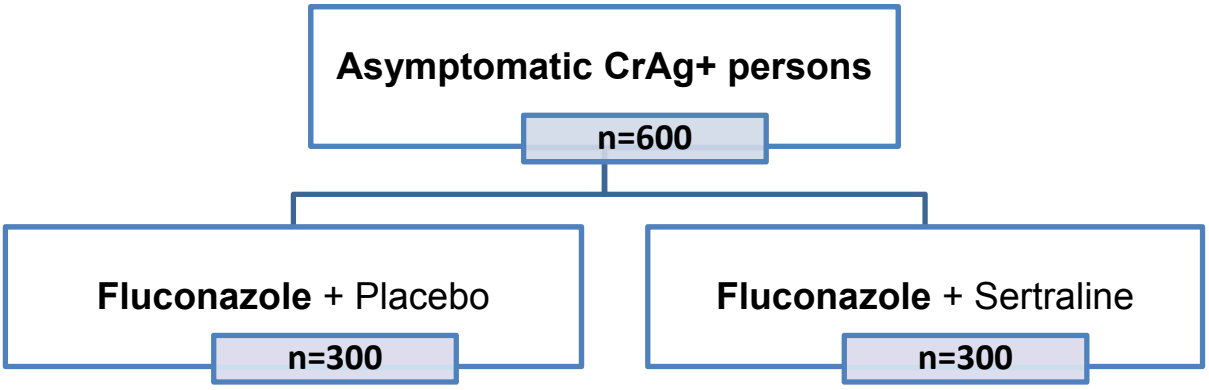
Symptomatic meningoencephalitis is defined as clinical symptoms consistent with meningitis AND

- CSF *Cryptococcus* culture positivity; OR
- CSF CrAg positivity; OR
- Cryptococcoma(s) by neuroimaging or post mortem exam.

6. STUDY DESIGN

This will be a randomized controlled trial of WHO standard of care with fluconazole vs. standard of care plus sertraline amongst asymptomatic CrAg+ persons in Uganda. There will be enhanced safety monitoring beyond usual standard of care with an integrated phase II / III study design. The first ~80 subjects enrolled will have additional real time safety monitoring and adjudication of unexpected adverse events or any potentially sertraline-related adverse events.

Study Design	Placebo	**Sertraline** x 16 weeks
Fluconazole 800mg/day x2 weeks, 400mg x 10 weeks; 200mg through 6 months (standard of care)	**~300 subjects**	**~300 subjects**



Outcomes of cryptococcal meningitis and survival will be assessed through 6 months from time of CrAg testing.

Participants may receive reminders via phone calls or text messages, to encourage them to return for appointments. Initially, during the phase II activities with enhanced safety monitoring, participants will have weekly contact through 6 weeks either in person (weeks 0, 2, 4, and 6) or by phone (weeks 1, 3, and 5).

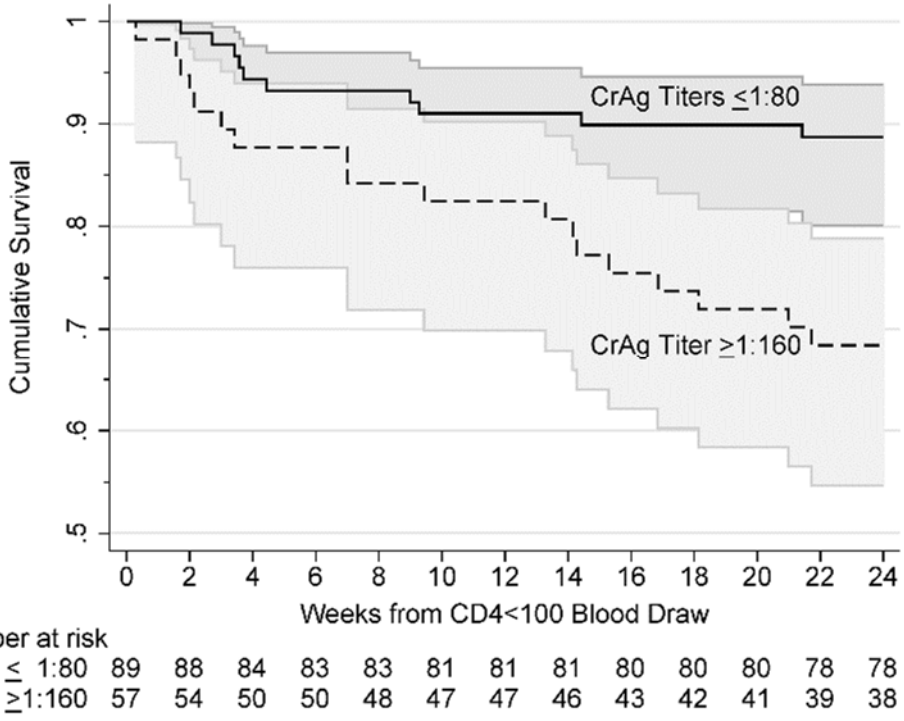
Objective 2: Identify risk factors for meningitis and death

The purpose of objective 2 is to define high risk populations that may require customized, intensive therapy in the future. We hypothesize that high initial CrAg titers in serum (≥1:160), high CRP, and other biomarkers are risk factors for meningitis and death. Preliminary data from the original ORCAS study in Uganda from 2012 to 2014 suggests that CrAg titer is a risk factor for death (see **Figure 7**).

In order to identify potential risk factors associated with treatment failure, we will perform CrAg titers at screening and among a subset at 4 and 8 weeks. A subset of samples (based on sample availability) will be stored and testing for biomarkers will be done

retrospectively in batches. Such biomarkers will include CRP, which we have previously demonstrated to be associated with IRIS and/or death. We anticipate that identifying biomarkers to predict meningitis and death, and validating titers as another risk factor, we will be able to use markers in the future to risk stratify patients prospectively, counsel them appropriately, and potentially customize therapy according to risk. Analysis may be performed retrospectively in batches.

Figure 7: Survival among CrAg+ Persons by Baseline Plasma CrAg Titer



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As part of exploring risk factors for meningitis and/or death, pathogenesis studies may include the following:.

1) Serum/plasma storage for CrAg titers, serum cytokines, and biomarker analysis;
2) Case-control exploratory sub-study on RNA transcriptome (gene expression) profiling will be performed on cases that fail preemptive therapy (i.e. develop cryptococcal meningitis or die), compared with controls with uneventful immune recovery with receipt of preemptive therapy. This case-control study will match controls with cases based on CrAg titer (+/- one two-fold dilution) and CD4 count (within 25 cells/μL). Participation in this sub-study would require an additional PaxGene RNA tube blood draw for storage at the enrollment visit with volume of 2.5 mL. Participants could decline participation in this sub-study and still participate in the trial.
3) PBMC collection and/or antigen stimulation experiments will occur at selected sites. This will be determined by site capacity, distance from central lab, and number of CrAg+ subjects at sites.

7. STUDY POPULATION

7.1. Participant Eligibility Criteria

This clinical trial will enroll HIV-infected CrAg+ persons at participating healthcare centers, who fulfill the inclusion/exclusion criteria outlined below. Cryptococcal infection is an AIDS-defining condition.

7.1.1. Inclusion Criteria

- HIV-infection, documented by any locally licensed rapid HIV test.
- Cryptococcal antigen positive test (CrAg+) in blood
- Age >=18 years and <65 years
- Provision of informed consent
- Female participants of childbearing potential who are participating in sexual activity that could lead to pregnancy must agree to use **one** reliable method of contraception: a barrier method of contraception (condoms or cervical cap) together with another reliable form of contraception (condoms, with a spermicidal agent; a diaphragm or cervical cap with spermicide; an intra-uterine device (IUD); or hormonal-based contraception) while receiving fluconazole ≥400mg/day.

7.1.2. Exclusion Criteria

- Prior history of cryptococcal meningitis
- Suspected meningitis (this is a clinical decision based on the patient's history and clinical findings, but typically is a combination of headache, neck stiffness, and/or altered mental status) or mania
- Suspected/known cirrhosis or serious hepatic comorbidities, transaminitis (ALT>5x upper limit of normal), jaundice, who should not receive fluconazole in the opinion of the study investigator
- Receiving an antidepressant medicine
- Currently receiving systemic antifungal therapy for >1 week
- Breastfeeding or Pregnancy (a negative urine (or serum) pregnancy test before study entry is required for women of childbearing potential).
- Contraindication to sertraline or fluconazole
- Currently receiving rifampicin (Rifampin®) or other prohibited medicine (Refer to Section 8.4)
- EKG QTc interval >450ms

Comments on exclusion criteria:

- Female participants of "childbearing potential" are defined as women who have reached menarche or who have not been post-menopausal for at least 24

consecutive months (i.e., who have had menses within the preceding 24 months) or have not undergone surgical sterilization (e.g., hysterectomy, or bilateral oophorectomy or salpingotomy). Abstinence is acceptable as a contraceptive method. Women are required to use at least one form of contraception while receiving fluconazole ≥400mg/day and are recommended to use contraception and avoid pregnancy while receiving 200mg/day.

- Persons with suspected symptomatic meningitis will be referred to hospital for diagnostic lumbar puncture and if CSF reveals cryptococcal meningitis (i.e. positive CSF CrAg+, culture, and/or India ink) amphotericin-based treatment will be initiated in accordance with WHO recommendations, outside of this study.
- Mania may be a presentation of cryptococcal meningitis (or bipolar disorder) and is an exclusion criterion.
- Prior ART is not an exclusion. ART-defaulters (i.e. prior receipt of ART who were lost to follow up and are now re-entering care) are not excluded. Persons with immunologic/virologic failure who may be switching to second line ART are eligible.
- Persons with a prior history of cryptococcal meningitis are excluded, as they may remain CrAg+ for months/years. In clinical practice, these persons should already be receiving fluconazole 200mg/day secondary prophylaxis if their CD4 is <100 cells/μL.
- CrAg positivity in "blood" includes: plasma, serum, whole blood, or by capillary fingerstick.[45, 46]
- ALT measurement within 30 days of randomization must occur for persons to be eligible. The ALT ideally could be run on a specimen collected at screening. If the result does not exist, potential participants should have an ALT collected prior to randomization. This may delay therapy and necessitate an extra return visit to the clinic. This is a directive from the NIH study sponsor.
- Rifampicin (rifampin) induces metabolism of sertraline via acting as an inducer hepatic enzymes of: CYP2B6, CYP2C9, CYP2C19, CYP3A4 (all relevant), as well as CYP2C8, CYP3A5, and CYP3A7 (not relevant to sertraline metabolism). In 9 Ugandans, mean sertraline levels with rifampin of 208 ng/mL (95%CI, 130 to 285) were 50% lower than sertraline levels without rifampin of 416 ng/mL (95%CI, 350 to 484; P<.001). Conversely fluconazole moderately inhibits CYP2C9 and CYP2C19 pathways. Refer to Table 10 in Section 8.6 for details on drug-drug interactions.
    - Rifapentine induces metabolism of CYP2B6, CYP3A4 (relevant) and CYP2C8 (not relevant).
    - Conversely, rifabutin is a weak inducer of CYP3A4 metabolism (relevant). Isoniazid is a CYP3A4 inhibitor.
- Prohibited medications are listed in Section 8.4. These medicines are not generally available in Uganda. Documentation of concomitant medicines will occur at study

entry, and updates will occur at study visits, as appropriate.

- A CD4 threshold is not an inclusion criterion as this is a trial of asymptomatic CrAg+ persons. The absolute CD4 count affects the probability of being CrAg+, but CD4 is immaterial once someone has early disseminated cryptococcal infection, as evidenced by a CrAg+ result in blood.
- EKG QTc >450ms exclusion is per the directive of the Funder. Refer to Section 8.1.7 on the published data on the negative "thorough QTc study" conducted on high dose sertraline as per FDA guidance.[33] Sertraline does not cause EKG QTc prolongation (FDA Package Insert).

7.2. Recruitment Process

CrAg screening in CD4<100 is recommended as per national HIV guidelines and is generally performed as a reflex-laboratory test whenever the CD4 is <100 cells/μL. Additional recommendations are to perform CrAg testing among persons with suspected virologic failure or WHO clinical stage III/IV disease.

In Kampala, the CrAg prevalence in persons with CD4<100 is 8-9% during 2015-2016. An estimated ~8000 persons will likely require CrAg screening to enroll a target of 600 asymptomatic CrAg+ persons. This protocol aims to enroll at approx. 125 participants per year irrespective of their CD4 count.

The protocol will not specify how clinics implement CrAg screening, as the screening method may vary by clinic based on clinic operations. SOPs will specify details of CrAg screening implementation. The probable methods of CrAg screening implementation are:

1) Lab-based reflex test using remnant plasma left over after CD4 testing
2) Provider initiated lab order
3) Point-of-care CrAg test in combination with point-of-care CD4 testing
4) Universal CrAg screening in the absence of prompt CD4 testing

The predominant method is likely to be lab-based reflex lab screening after CD4 testing at the CD4 laboratories for the sites. Refer to Appendix C for the overview of specimen collection and flow of laboratory testing by site.

Positive CrAg tests are considered a critical laboratory value, which results in a phone call from the laboratory to the ordering provider. Study staff notified of positive CrAg tests will contact CrAg-positive patients to help facilitate persons returning to clinic expeditiously. CrAg-positive persons identified by lab-reflex screening by clinic staff (through point-of-care testing), or via referral from outside clinics will be reviewed for eligibility and approached for informed consent by study personnel (typically a study nurse). Asymptomatic CrAg+ persons who meet inclusion criteria will be offered enrollment in this study.

Persons referred from outside, non-study clinics must have a CrAg test repeated by the study laboratory or by trained study personnel prior to randomization (refer to the Delegations and Responsibility log as to which investigators have been trained).

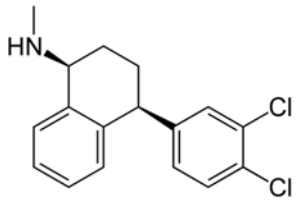
All participants must be verified for CrAg positivity at a DAIDS-approved facility prior to randomization.

8. INTERVENTIONS (STUDY PRODUCT)

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

8.1. Biomedical Interventions

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.



Ample clinical data supports the safety of sertraline at doses between 50-200mg.[47-49] Few drug-drug interactions of clinical significance exist[50], and it is relatively safe in over-dosage.[42, 43] 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).[51] 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.[52] Akathisia caused by sertraline was observed in 16% of patients in a case series.[53] 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.[43] 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.[54] 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.[55]

All antidepressants, including sertraline, carry an FDA black box warning stating that antidepressants may increase the risk of suicide in persons with depression 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.[56] Considered separately, sertraline use in adults decreased the odds of

suicidal behavior with a marginal statistical significance by 37% or 49% depending on the statistical technique used.[56]

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.[57] 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.[58]

In the 2013-2014 pilot study of the “Adjunctive Sertraline for the Treatment of Cryptococcal Meningitis” (ASTRO-CM) open-label pilot study in treating patients with symptomatic cryptococcal meningitis, the incidence of adverse events was not increased with higher dose sertraline.[33] **Table 4** compares the incidence of AEs among persons with cryptococcal meningitis who were treated with a background regimen of amphotericin B deoxycholate 0.7-1.0 mg/kg/day and fluconazole 800mg/day (COAT Trial 2010-2012 in Uganda) and that background regimen plus sertraline at doses 100-400mg/day.

Table 4: Safety Endpoints of Adjunctive Sertraline used for cryptococcal meningitis

Clinical Outcomes	Historical COAT trial cohort [35]	ASTRO Pilot Sertraline 100 and 200mg/day[33]	ASTRO Pilot Sertraline 300 and 400mg/day[33]	Risk Difference (95%CI) [a]	*P*-value
N enrolled	208	77	95		
Adverse Events					
Total number Grade 4 AEs	119	20	27		
Grade 4 AEs, cumulative incidence	88 (50%)	15 (19%)	22 (23%)	0.04 (-0.09, 0.16)	0.56
Total number Grade 5 AEs		3	9		
Grade 5 AE, cryptococcal related	N/A	1 (1%)	3 (3%)	0.02 (-0.02, 0.06)	0.42
Grade 5 AE, non-cryptococcal related	25 (12%)	2 (2.6%)	5 (5.2%)	0.03 (-0.03, 0.08)	0.38
Overall 12-week Mortality					
Cryptococcal-related mortality	60 (29%)	19 (25%)	23 (24%)	0.0 (-0.13, 0.12)	0.94
Non-cryptococcal related mortality	33 (16%)	11 (14%)	16 (17%)	0.03 (-0.08, 0.13)	0.65
Cryptococcal Relapse	3 (1.7%)	0 (0%)	0 (0%)	--	--
Cryptococcal Paradoxical IRIS Cumulative Incidence[*]	15 (9.6%)	1 (6.7%)[*]	1 (4.3%)[*]	1.27 (0.69, 2.32)[§]	0.45[§]
Nausea, Vomiting, or Diarrhea, ≥1 event	142 (68%)	59 (77%)	61 (64%)	-0.12 (-0.26, 0.01)	0.08
Serotonin Syndrome	0 (0%)	0 (0%)	1 (1%)[**]	--	--
ALT >5x ULN (Grade 3)	3 (1.7%)	2 (2.6%)	0 (0%)		
T-Bilirubin >5x ULN (Grade 3)	1 (0.6%)				
Premature sertraline dose reduction, all cause	--	0 (0%)	1 (1%)	--	--
Premature sertraline discontinuation	--	1 (1%)	5 (5%)	0.04 (-0.01, 0.09)	0.16
Lost to Follow up	0%	3 (4%)	2 (2%)	-0.02 (-0.07, 0.03)	0.49

After two weeks of sertraline induction therapy in combination with amphotericin and fluconazole 800-1200mg/day, all participants received 200mg/day until week 8 and then tapered through week 11. The historical 2010-2012 cohort only included ART-naïve persons who received amphotericin and fluconazole 800mg/day of whom 31 died before randomization, and 177 were randomized into the COAT trial.[35]

[a] Risk differences of 300 and 400mg/day versus 200 and 100mg/day. No statistical comparisons to the historical cohort were performed.

[*] Percentages calculated from competing risk model for paradoxical cryptococcal-IRIS among patients without prior history of cryptococcal meningitis, who were ART-naïve and who survived to initiate ART during the trial period. (N=15 for 100 and 200mg/day; N=23 for 300 and 400mg/day.) Passive follow up

after 12 weeks identified 1 case of relapse in a 100mg/day participant after sertraline discontinuation. In COAT trial, 7 additional relapse episodes and 11 additional IRIS events occurred after 12 weeks through 48 weeks

§ Hazard ratio and 95% CI from competing risks regression and corresponding p-value.

** Protocol deviation by a participant taking 800mg/day for three days.

8.1.1. Regimen (dose, schedule, route, administration)

Study administration: Patients enrolled in this study will receive sertraline or placebo, as outlined in Section 6. This trial will use sertraline 100 mg tabs or masked placebo (**Table 5**).

Table 5: Schedule of study medicine to be dispensed, tablets per day.

Study Week	1	2	3	4-12	13	14	15	16
Placebo Control, tabs	1	2	3	4	3	2	1	0
Sertraline, tabs	1	2	3	4	3	2	1	0

Note: Each sertraline tab is 100mg tablet

The use of sertraline (or placebo) is divided into three phases. During the escalation phase (the first 3 weeks), subjects will initially receive 100mg (1 tab) dose of sertraline/placebo for days 1-7 increasing to 200mg/day (2 tabs) for days 8-14. After receiving 14 days, subjects will escalate to sertraline/placebo of 300mg/day (3 tabs) for days 15-21. After 21 days, subjects will increase to 400mg/day (4 tabs) on day 22. This escalation in dose will be facilitated via a blister pack of study medications with doses of placebo/sertraline apportioned for the first ~30 days. The 4 tabs daily will be continued through the week 12 visit. Sertraline (or masked placebo) will begin to be tapered off starting after week 12. The taper will entail taking 300mg (3 tabs) daily for 1 week, then 2 tablets daily for 1 week, and finally 100mg (one tablet) daily for 1 week. This taper will also be apportioned via a blister pack of study medications. Participants will be off of all study drugs after week ~15. The tapered discontinuation is intended to avoid a withdrawal syndrome.

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%.[37] ART will be initiated at ~2-4 weeks and/or second line ART will be changed at 2-4 weeks.

8.1.2. Rationale for Sertraline Dose Selection

Sertraline is typically administered at doses of 50mg-200mg for depression. Doses above this threshold do not have any further efficacy for depression treatment, but there is not additional toxicity. Our group has studied sertraline in the treatment for symptomatic cryptococcal meningitis at doses of 100mg to 400mg.[33] Notably, grade 4

or 5 adverse event risk did not differ between dosing of 100-200mg/day vs higher doses of 300-400mg/day.

Prior studies have reported that efavirenz lowers serum sertraline levels by 39%.[37] In our Ugandan meningitis study, we found that the presence of any ART (e.g. efavirenz, nevirapine, or PIs) significantly increased sertraline clearance lowering the serum levels by ~50%.[33] This effect is no longer seen at sertraline doses of 400mg/day. Thus ART-status does not appreciably affect sertraline levels at this 400mg daily dose.

Table 6. Mean Steady State Sertraline Plasma Concentrations at Days 7-14.

Dose Cohort	**Percentiles Sertraline Concentration** (ng/mL)						
	5	10	25	50	75	90	95
200mg on ART (n=26)	22	26	64	101	214	336	675
200mg ART naïve (n=24)	108	122	198	257	362	512	532
300mg on ART (n=23)	43	67	111	194	305	367	458
300mg ART naïve (n=23)	43	126	201	298	392	577	673
400mg on ART (n=13)	139	146	262	395	486	722	
400mg ART-naïve (n=18)	118	128	278	414	583	679	

Figure 8. The Distribution of Sertraline Plasma Concentrations by ART Status

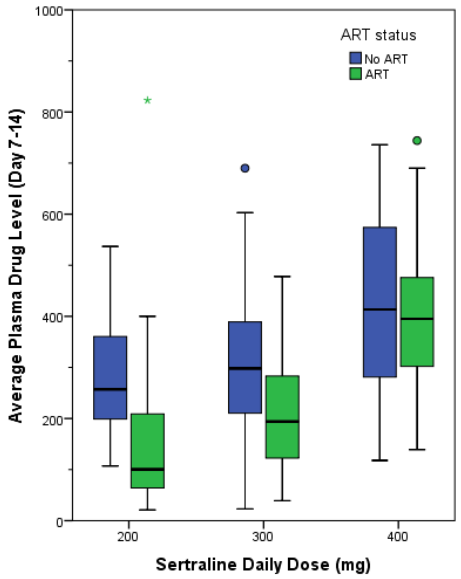


Figure 8 provides a visual distribution of **Table 6** data based on steady state plasma concentrations measured between day 7 and day 14. This is based on 423 total measurements among 140 research participants of whom 127 received sertraline doses of 200-400mg/day in conjunction with fluconazole 800mg/day.

Figure 9 displays the projected proportion achieving therapeutic levels of sertraline in the brain by dose and ART status based on *Cryptococcus* organism susceptibility.[33] Notably those on ART achieved lower brain concentrations compared to those not on ART. [33] At 400mg per day, highest brain concentrations of sertraline are achieved. In general, there is bi-directional synergy between sertraline and fluconazole, with a two-fold reduction in MIC (i.e. sertraline MIC=4 μg/mL in the presence of fluconazole is

lowered to ~2 μg/mL as well as the fluconazole MIC being lowered 2-fold). As per Table 2, ~85% of *Cryptococcus* isolates have a MIC ≤4 μg/mL.

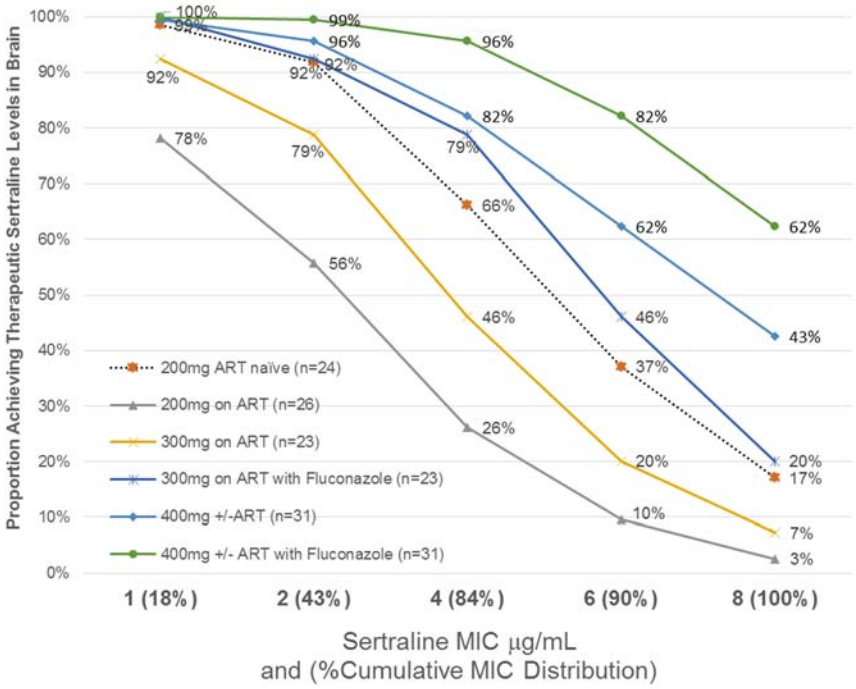
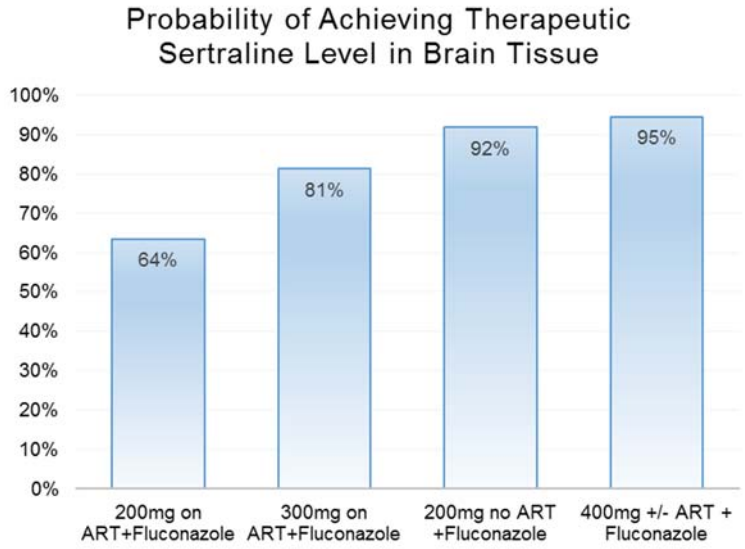


Figure 9. Projected Proportion with Therapeutic Sertraline Levels in Brain based on Sertraline Dose, ART Status, and *Cryptococcus* MIC Susceptibility.



Based on the distribution of *Cryptococcus* MICs and tissue penetration,[33, 59] we project in the presence of 2-fold additive/synergistic effects of fluconazole, 92% of participants receiving sertraline at 200mg/day without ART and 81% of participants receiving 300mg/day with ART will achieve therapeutic concentrations of sertraline in the brain. With

400mg daily of sertraline, an estimated 95% will achieve therapeutic drug levels in the brain, regardless of ART status. Steady state levels in blood are achieved by 7 days of sertraline dosing.[33]

Based on the average fold-change increase over blood, based on post-mortem studies,[59] therapeutic sertraline levels should be achieved by 100% of participants in liver, lung, spleen, and bile (**Table 6**).

Table 6. Fold-Change Increase in Sertraline Tissue Concentration over Blood

	Liver	Lung	Kidney	Spleen	Muscle	Brain	Heart	Bile
Average	86.2	68.6	7.4	45.8	2.1	21.7	8.9	32.0
±SD	92.5	49.8	5.3	47.0	1.3	14.5	7.8	28.4
Min	12.5	11.0	3.7	7.0	0.9	10.0	1.6	0.0
25th Percentile	26.9	22.8	4.3	17.3	1.0	13.0	3.8	7.8
Median	46.4	64.7	5.2	34.6	1.5	16.5	5.3	20.8
75th Percentile	102.7	102.4	6.8	48.0	3.5	21.3	13.0	52.4
Max	322.3	157.8	18.4	167.2	4.1	57.3	22.6	78.3

8.1.3. Study Product Formulation and Preparation

A U.S. FDA-approved sertraline manufacturer will be used (e.g. NorthStarRx, Memphis, TN) to provide Sertraline 100mg film-coated tablets.

A matched placebo will be prepared in Uganda by Kampala Pharmaceuticals Industries, Ltd. located at Plot M444B / P.O Box 7551 Kampala, Uganda. A finished product certificate of analysis will be provided to certify assurance testing that the placebo matches in physical appearance, size, average weight, disintegration time, and that the placebo lacks sertraline.

8.1.4. Study Product Supply and Accountability

Product Storage and Stability: Sertraline will be stored at room temperature (25ºC) with excursions permitted to 15ºC-30ºC (59ºF to 86ºF) according to manufacturer guidelines. Placebo may be stored at room temperature with excursions permitted to 15ºC-35ºC.

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. The pharmacist of record at the Infectious Diseases Institute will be the central pharmacist.

Compliance with the randomization will be assessed via ongoing quality assurance monitoring via CRF reporting of the dispensed medication (e.g. unique identifier) and the DataFax management system.

8.1.5. Sertraline Side Effect Profile

The most common treatment-emergent adverse events associated with the use of sertraline in placebo-controlled clinical trials in adult patients with major depressive disorder, obsessive-compulsive disorder, panic disorder, posttraumatic stress disorder, premenstrual dysphoric disorder, and social anxiety disorder (FDA Package Insert) are generally minor and not dose dependent. Table 7.

BODY SYSTEM/ADVERSE EVENT	ZOLOFT (N=2799)	PLACEBO (N=2394)
Autonomic Nervous System		
Ejaculation Failure (males)	14 %	1 %
Mouth Dry	14 %	8 %
Sweating Increased	7 %	2 %
Central & Peripheral Nervous System		
Somnolence	13 %	7 %
Insomnia	21 %	11 %
Dizziness	12 %	7 %
Headache	25 %	23 %
Paresthesia	2 %	1 %
Tremor	8 %	2 %
Disorders of Skin		
Rash	3 %	2 %
Gastrointestinal		
Anorexia	6 %	2 %
Constipation	6 %	4 %
Diarrhea / Loose Stools	20 %	10 %
Dyspepsia	8 %	4 %
Nausea	25 %	11 %
Vomiting	4 %	2 %
General		
Fatigue	12 %	7 %
Psychiatric Disorders		
Agitation	5 %	3 %
Anxiety	4 %	3 %
Libido Decreased	6 %	2 %
Nervousness	5 %	4 %
Special Senses		
Vision Abnormal	3 %	2 %

All other adverse events were <2% and/or of equal or greater frequency with placebo.

8.1.6. Sertraline Precautions

Liver Disease. Sertraline is metabolized by the liver. As per the FDA package insert, “Liver impairment can affect the elimination of sertraline. In patients with chronic mild liver impairment (N=10, 8 patients with Child-Pugh scores of 5-6 and 2 patients with Child-Pugh scores of 7-8) who received 50 mg sertraline per day maintained for 21 days, sertraline clearance was reduced resulting in approximately 3-fold greater exposure compared to age-matched volunteers with no hepatic impairment (N=10).” “The use of sertraline in patients with liver disease must be approached with caution. If sertraline is administered to patients with liver impairment, a lower or less frequent dose should be used.”

Hepatotoxicity. The FDA package insert lists hepatotoxicity, from double-blind placebo controlled trials as a “rare event,” defined as <1 in 1000 persons.[51]

Specifically in regards to hepatotoxicity, during the ASTRO-CM pilot study (n=177) in Uganda,[33] two participants (1%) developed new ALT/AST elevation while receiving sertraline 200mg/day and fluconazole 400mg/day (and no ART). The first participant at 6 weeks had a serum ALT = 152 U/L (grade 2) and AST = 217 U/L (grade 3), which was attributed to alcohol. The patient was counseled, and re-assessment 4 and 6 weeks later revealed resolution (ALT = 67 then 26 U/L, AST =70 then 29 U/L). No medications were changed. A second participant, at 4 weeks had ALT = 402 U/L (Grade 3) and AST = 346 U/L (Grade 3). Follow up re-check 5 days later (ALT = 100 U/L and AST 44 U/L) and one month later (ALT = 33, AST = 56) demonstrated resolution. No medications were changed.

Sertraline plasma concentrations at 4 weeks were 433 and 228 pg/mL, respectively for these two participants, equating to ~80th and ~35th percentile for sertraline dosed at 200mg/day.

Outcomes among persons with baseline liver transaminase elevation have also been documented in HIV-infected Ugandans living with AIDS during the ASTRO-CM pilot. One participant had <u>baseline</u> asymptomatic Grade 4 ALT/AST elevations (ALT = 544, AST = 918 U/L), which steadily declined over two weeks while receiving sertraline 400mg/day and fluconazole 800mg/day. After 14 days, the ALT = 92 and AST = 117 U/L. For this participant, plasma steady state levels between days 10-14 averaged 157 pg/mL (~20th percentile for 400mg/day dose group).

Thus, baseline or new liver transaminase elevations did not correspond to elevated plasma sertraline levels. Elevation of transaminases is not diagnostic of cirrhosis or liver impairment.

Renal Disease. As per the FDA package insert, excretion of unchanged drug in urine is a minor route of elimination. “In volunteers with mild to moderate (CL_{CR}=30-60 mL/min), moderate to severe (CL_{CR}=10-29 mL/min) or severe (receiving hemodialysis) renal impairment (N=10 each group), the pharmacokinetics and protein binding of 200 mg sertraline per day maintained for 21 days were not altered

compared to age-matched volunteers (N=12) with no renal impairment. Thus sertraline multiple dose pharmacokinetics appear to be unaffected by renal impairment."

Substantial Adverse Reactions listed in the FDA Package insert with <1 per 1000 incidence.

Hyponatremia. "Hyponatremia may occur as a result of treatment with SSRIs and serotonin–norepinephrine reuptake inhibitors, including <sertraline>. In many cases, this hyponatremia appears to be the result of the syndrome of inappropriate antidiuretic hormone secretion (SIADH). Cases with serum sodium lower than 110 mmol/L have been reported. Elderly patients may be at greater risk of developing hyponatremia with SSRIs and serotonin–norepinephrine reuptake inhibitor. Also, patients taking diuretics or who are otherwise volume depleted may be at greater risk. Discontinuation of <sertraline> should be considered in patients with symptomatic hyponatremia."[51]

Platelet Function. **"**There have been rare reports of altered platelet function and/or abnormal results from laboratory studies in patients taking <sertraline>. While there have been reports of abnormal bleeding or purpura in several patients taking <sertraline>, it is unclear whether <sertraline> had a causative role.[51]

Serotonin Syndrome. "Patients should be cautioned about the risk of serotonin syndrome with the concomitant use of serotonin–norepinephrine reuptake inhibitors and SSRIs, including <sertraline>, and triptans, tramadol, or other serotonergic agents," such as tricyclic antidepressants, fentanyl, lithium, tryptophan, buspirone, and St. John's Wort. These medications are listed as prohibited medications in Protocol Section 8.4. Manifestations of serotonin syndrome include: confusion, agitation, reduced level of consciousness, seizures, myoclonus/clonus, hyperreflexia, tremors, muscle rigidity, ataxia, akathisia, hyperthermia, hypertension, tachycardia, diaphoresis, lacrimation, mydriasis, shivering, and/or diarrhea.

Acute Angle Closure Glaucoma is an acute event associated with nausea, vomiting, and acute eye pain. The more common pre-existing glaucoma would be open-angle glaucoma. The incidence of angle-closure glaucoma increases with age, is more common in women, and has a greater prevalence in Asians and East Indians than in Europeans and African. The prevalence of angle-closure glaucoma in the South Africa Black population was 2 per 100,000 per year. Most cases of primary angle closure glaucoma occur in the 6th to 7th decade of life.

8.1.7. Electrocardiogram QT Interval Safety – Published Data

Prolonged QT interval is considered a class effect seen with some SSRIs, particularly with citalopram (Celexa) and escitalopram (Lexapro). However, of the SSRIs, sertraline has the least effect on QT interval.[43] The FDA package insert states, "the electrocardiograms of 774 patients who received ZOLOFT in double-blind trials

were evaluated and the data indicate that ZOLOFT is not associated with the development of significant EKG abnormalities." A meta-analysis of 4292 adults receiving any SSRI, reported an average 3ms increase in QTc interval among persons receiving sertraline.

In Uganda, during electrocardiogram QT monitoring of 53 research participants over 2 weeks with cryptococcal meningitis in Uganda receiving sertraline + fluconazole 800mg/day + amphotericin ± ART, there was no evidence for QT prolongation (**Table 8**).

The mean change in QTc is -23ms (95% CI +0.75 to -45.2ms, P=0.043 by paired t-test) from baseline to day 14 of fluconazole 400mg/day and various doses of sertraline (n=29 pairs). Among those receiving 300-400mg/day, there was no dose-dependent effect (P=0.56), and overall the mean QT_c interval had a greater shortening with the higher doses (i.e. -28ms change with 300-400mg/day vs. -14ms change with 100-200mg/day).

Table 8. Mean electrocardiogram QTc Interval with adjunctive sertraline therapy and fluconazole 800mg/day in persons with cryptococcal meningitis at Mulago Hospital, Uganda.

	N	Day 1 QTc (ms)	N	Day 7 QTc (ms)	N	Day 14 QTc (ms)	P-value
Sertraline Dose							
100–200mg/day	25	381 (367, 395)	23	391 (383, 399)	18	367 (343, 390)	0.56
300–400mg/day	29	383 (372, 394)	26	392 (368, 416)	18	355 (331, 380)	
300mg/day	13	384 (367, 401)	11	393 (381, 406)	8	340 (288, 393)	0.33
400mg/day	16	382 (366, 399)	15	390 (347, 434)	10	367 (343, 391)	
Sex							
Men	39	383 (373, 394)	35	389 (371, 407)	27	369 (352, 385)	0.35
Women	15	379 (363, 395)	14	396 (386, 406)	9	337 (291, 383)	
ART at Diagnosis							
Receiving ART	24	377 (361, 393)	23	399 (373, 424)	13	365 (340, 391)	0.50
Not receiving ART	30	386 (377, 396)	26	385 (373, 396)	23	358 (336, 381)	

Data displayed as mean (95%CI). P-values testing for interaction between follow-up time and sertraline dose group, sex, and ART at diagnosis, from linear mixed model.

No ASTRO-CM trial participants developed a new QTc >450ms (Grade 1 DAIDS Toxicity) while on sertraline, fluconazole 800mg/day, and amphotericin.

8.1.8. Sertraline Safety in Overdose

SSRIs have a wide therapeutic margin of safety.[43, 44]

Among a published case series of 469 SSRI intentional overdoses among 156 persons taking sertraline alone, overdosing on a median of 24x daily dose (IQR 16-30) of 200mg/day, toxicity (i.e. median ingestion = 4800mg).[43] Among this sertraline group: 2% (n=3) developed self-limited seizures and 20% (n=31) serotonin syndrome. There were no cases of severe serotonin syndrome requiring ICU admission. Another 3% (n=4) presented with a GCS<9, but this associated with co-ingestion of sedative medications. Of 310 overdoses, solely of SSRI medicines, with EKGs obtained, the following table demonstrates cardiac toxicities observed as median (IQR).[43]

Table 9. Cardiovascular Changes with SSRI Overdoses

Parameters With Overdose	Sertraline (Zoloft®)	Paroxetine (Paxil®)	Fluoxetine (Prozac®)	Citalopram (Celexa®)	ASTRO Pilot [33]
N	103	78	42	57	26
Age, years	28 (20-37)	31 (23-28)	35 (23-41)	30 (22-41)	37 (31-42)
Daily Defined Dose ingested of 200mg	24x (16-30)	15x (10-28)	16x (10-26)	16x (10-25)	1.5-2x
Heart Rate	82 (68-97)	78 (68-90)	82 (71-100)	77 (68-90)	
Hypotension, systolic <90 mmHg	0	0	0	2 (3.5%)	0
Arrhythmia	0	0	0	1 (1.8%)	0
EKG QT, ms	380 (360-400)	375 (350-400)	380 (360-400)	400 (360-440)	302 (261-337)
EKG QTc, ms	429 (413-456)	427 (409-454)	432 (412-466)	450 (436-484)	382 (319-397)
EKG QTc >500ms	6 (6%)	1 (1%)	4 (10%)	7 (12%)	0 (0%)

In a comparison group (n=309) ingesting non-cardiac toxic medications at the same site, the median QTc was 423ms (IQR, 404-447) versus the QTc of 429 (IQR, 413-456) in sertraline overdose.[43]

The wide therapeutic safety margin is evidenced by the existence of evidence-based consensus guideline for out-of-hospital management for SSRI poisonings, based on the review of 48,000 SSRI exposures at U.S. Poison Centers in the year 2004.[44]

For asymptomatic patients or those with mild effects following acute SSRI ingestions of sertraline of up 1250mg can be observed at home without any medical evaluation.[44] Mild

effects were defined as vomiting, mydriasis, diaphoresis, or somnolence (but arousable with speaking voice or light touch).[44]

8.2. Fluconazole

Fluconazole will be provided via the clinic as standard of care.[13] Interruptions in care resulting from supply stock outs of standard therapies will be recorded and taken into account in the analyses.

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 ≥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 is a standard of care medicine which may be obtained from the Ministry of Health, directly from the Diflucan Partnership Program (which in 2016 has a very intermittent supply), or any nationally registered or WHO prequalified fluconazole supply is acceptable.

Product Storage and Stability: Fluconazole needs to be stored below 86°F (30°C) and above 41°F (5°C). Storage shall be per the manufacturer's recommendation.

Study administration: Fluconazole is an oral medication, used for the treatment of many fungal infections including cryptococcosis. Dosing in this study will be per Ugandan and WHO standard of care.[13]

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).

Side Effects: FDA Diflucan® label states: "Clinical adverse events were reported more frequently in HIV infected patients (21%) than in non-HIV infected patients (13%); however, the patterns in HIV infected and non-HIV infected patients were similar. The proportions of patients discontinuing therapy due to clinical adverse events were similar in the two groups (1.5%). The following treatment-related clinical adverse events occurred at an incidence of 1% or greater in 4048

patients receiving DIFLUCAN for 7 or more days in clinical trials: nausea 3.7%, headache 1.9%, skin rash 1.8%, vomiting 1.7%, abdominal pain 1.7%, and diarrhea 1.5%."

8.3. Assessment of Participant Adherence

Pharmacy will record the dispensing history for the study medicine.

Reconciliation of all study medicines will be performed by pharmacy staff (or their designee) via pill counts at time of dispensing and/or collection of returned study medicines.

Study medical officers will query self-reported adherence at outpatient visits to clarify participant understanding of the correct dosing and/or the number of missed study medicines since the last visit. Similar self-reported assessment of fluconazole and ART adherence will occur.

Adherence to the exact taper will not be assessed at week 16 as no study medicines should be left. Pharmacy reconciliation will however occur if there are leftover study medicines at the week 16 visit.

8.4. Prohibited Medications

Monoamine oxidase inhibitors (MAOIs) and pimozide are contraindicated. These are not registered nor available in Uganda. These are antidepressants and an exclusion criterion.

Other prohibited medications include serotonergic drugs: sumatriptan, zolmitriptan, tricyclic antidepressants, fentanyl, lithium, tramadol, tryptophan, buspirone, and St. John's Wort. None of these medications are commonly available in Uganda (i.e. not available nor prescribed).

A medication log will record all medications/substances taken by participants

Below is a list of precautions taken by clinicians, per standard of care.

8.5. Concomitant Medications and Procedures

Standard HIV care shall be prescribed as part of routine care outside of this study.

1. 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.[37] Efavirenz is first line ART. In Uganda, we found a ~50% reduction in the area under the curve (AUC) exposure at lower doses of sertraline 100-300mg/day.[33] However, at sertraline doses of 400mg daily, this effect was no longer seen, with non-statistical differences in plasma levels.[33] Thus, there will be no dose adjustment based on antecedent ART-status.

2. Darunavir/ritonavir combination also decreases the sertraline AUC by ~49% (90%CI, 42%-54%) and C_{max} by ~44% (90%CI, 37%-51%) (Prezista FDA Package insert). There is a paucity of published data on other protease inhibitors, but with the similar effect observed with darunavir/ritonavir, persons on second-line ART using protease inhibitors will also receive the same dosing schedule of the study medicine (sertraline vs. placebo). The lower AUC is likely due to the induction of metabolism by ritonavir of hepatic P450 enzymes of CYP2B6 (33-50%), CYP2CP (33-40%), and CYP2C19.[62]
3. If steroids are to be prescribed, prednisolone would be preferred with avoidance of dexamethasone which induces the metabolism of sertraline.
4. Rifampin enhances the metabolism of concurrently administered fluconazole, decreasing the AUC by 23%.

   > FDA Package Insert: "Administration of a single oral 200 mg dose of DIFLUCAN after 15 days of rifampin administered as 600 mg daily in eight healthy male volunteers resulted in a significant decrease in fluconazole AUC and a significant increase in apparent oral clearance of fluconazole. There was a mean ± SD reduction in fluconazole AUC of 23% ± 9% (range: –13 to –42%). Apparent oral clearance of fluconazole increased 32% ± 17% (range: 16 to 72%). Fluconazole half-life decreased from 33.4 ± 4.4 hours to 26.8 ± 3.9 hours."
   >
   > U.S. DHHS HIV OI Guidelines recommend: "Monitor for antifungal efficacy; may need to increase fluconazole dose."[63]

5. Rifampin may also enhance the metabolism of sertraline, but this is not clearly known. During the ASTRO pilot using sertraline,[33] minimal drug-drug interaction was observed. Rifampin at time of study entry is an exclusion criterion; however, if persons are diagnosed with TB after study entry and require TB therapy, participants shall remain in the C-ASSERT trial.
6. 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 should be used, which is in accordance to national guidelines.

   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.[43] Nevertheless, a "thorough QT/QTc study" has been conducted to assure safety, as per FDA guidance.[64] Among participants in the ASTRO-CM pilot study who received sertraline up to 400mg/day and fluconazole 800mg/day. The mean QTc interval decreased by -23ms (95%CI, +0.75 to -43ms, P=0.04) overall.[33] There was no difference in QTc change between 100-200mg/day doses and 300-

400mg/day doses (P=0.56) with higher doses trending toward greater decrease.[33] This is a “negative thorough QT/QTc study” based on the upper 95%CI <10ms by FDA criteria.[64] In the ASTRO-CM study, the upper 95%CI was +0.75ms.[33] At time of higher sertraline doses of 300-400mg/day, fluconazole will be dosed at 400mg/day, which does not have an appreciable QTc effect.

7. 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 with the repeated scheduled CBCs at 4, 8, and 12 weeks.
8. 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. Participants in our study will not be started on protease inhibitors while still receiving high-dose fluconazole. If participants are to be started on second line ART, participants should be started on protease inhibitors after reduction to <=400mg/day of fluconazole after 2 weeks (i.e. switching to second line ART should occur at/after 2 weeks).
9. Nevirapine: Fluconazole is known to increase the concentration of Nevirapine.[65] If administered together, participants will be closely monitored for nevirapine-associated adverse events. Healthcare providers will be encouraged to prescribe an efavirenz-based ART regimen if possible, given drug interactions between nevirapine and fluconazole. Nevirapine is no longer a first line ART medicine. 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. [66] Thus nevirapine is not contraindicated; however, efavirenz remains preferred.

8.5.1. Rescue Medications

Persons developing symptomatic meningitis shall be referred to hospital for appropriate management, which should include amphotericin B based combination therapy for culture-positive meningitis.

8.6. Pharmacokinetic Interactions between Sertraline, Antiretrovirals, and Fluconazole.

Sertraline has relatively few drug-drug interactions as it is metabolized by five different cytochrome p450 pathways. Antiretrovirals appear to be the exception to the rule as both NNRTI and PIs induce multiple pathways of metabolism (Table 10).

Drug	Table 10. Sertraline Hepatic P450 Metabolism Pathways (% of sertraline metabolism)				
	CYP2B6 (36-40%)	**CYP2C9 (15-17%)**	**CYP2C19 (4-14%)**	**CYP3A4 (15-18%)**	**CYP2D6 (16%)**
Efavirenz	Induction varies by genetic polymorphism,[67] ~55% at 400 mg.[68]	Moderate Inhibitor, increase EFV 1.7-2 fold at steady state [69]	Inducer,[70] dose dependent, ~20% at 3 mcg/mL EFV[69]	Inducer [70] ~20%[69]	Negligible [71]
Nevirapine	Inducer,[72] with 20-50% decrease AUC [73]	No effect [71]	No effect [71]	Weak Inducer [72, 74]	Negligible [71]
Dolutegravir	No effect [75]	No effect [75]	No effect [75]	No effect [75]	No effect [75]
Lopinavir/r	Negligible [76, 77]	29% induction by LPV/r[78]	100% induction by LPV/r[78]	CYP3A Substrate / Weak inhibitor[78]	Negligible at clinically relevant LPV/r levels[79]
Atazanavir	No effect [80]	Negligible Inhibition at clinically relevant doses[80, 81]	Negligible Inhibition at clinically relevant doses [80, 81]	Substrate / Weak inhibitor[82]	No effect[80]
Darunavir/r*	Induction [80]	Induction [80]	Induction [80]	Inhibition by DRV/r [80]	No effect [80]
Ritonavir	Induction in vivo [62] 33-50%	Induction[62] ~33-40%	Induction [62]	Strong Inhibitor[76]	Inhibitor[62]
Cobicistat	Negligible [83]	Negligible [83]	Negligible[83]	Strong Inhibitor[84]	Inhibitor[85]
Fluconazole (at 400mg)	Negligible [86]	Modest Inhibitor[73, 87] ~40% in vitro	Moderate Inhibitor[73, 87] ~70% in vitro	Negligible [86, 87]	Negligible [86]

* darunavir/ritonavir reduces sertraline AUC by 49%.[88]

Existing Ugandan sertraline PK data[33] were collected with concomitant fluconazole 800mg/day. There is dose dependent inhibition of hepatic P450 CYP2C9 and CYP2C19 pathways by fluconazole which accounts for 20-30% of sertraline metabolism. Ritonavir also induces and inhibits pathways of sertraline metabolism. Overall, ritonavir induces pathways accounting for 70% of sertraline metabolism and inhibits pathways accounting for 40% of sertraline metabolism. The net effect when studied with Darunavir/ritonavir was a 49% reduction in the sertraline AUC.[88] This is similar as to the PK effect observed in Uganda with NNRTI regimens of efavirenz or nevirapine.[33] Thus, we would expect a similar PK effect on sertraline with any ART regimen, most likely.

9. STUDY PROCEDURES / EVALUATIONS

9.1. Schedule of Events

Event	CRAG Screen	Pre-Entry	Enroll	2w	4w	8w	12w	16w	20w	24w	Sick Visit
CD4 Count	*Routine Care*										
CrAg	X										
Review Eligibility		X									
Informed Consent		X									
Medical History		X									
Detailed Study Visit			X	X	X	X	X	X	X	X	X
Mental Health PHQ-9			X		X	X	X				
Assess for AEs			X	X	X	X	X	X	X	X	X
CrAg Titer	X		±*			±*					
Pregnancy Test		♀X									±♀
Lab Monitoring †		ALT	X		X	X	X				X
EKG Monitoring		X			X	X	X				
Fluconazole, mg			800	400	400	400	200	200	200		
Randomization			X								
Study medicine, tabs			1 → 2	3→4	4	4	*Taper*	0	0	0	
ART Initiation/Switch				*Target*	*Target*						
Specimen Storage*	X		X		X	X	X				X

*Pathogenesis studies including CrAg titer, peripheral blood mononuclear cell (PBMC) collection, RNA collection (2.5mL), and/or antigen stimulation may occur at selected clinical sites with the capacity to store these samples (Refer to Appendix C). Approximately 40mL of blood will be drawn in total for research purposes during the study, after consent. Refer to laboratory SOPs for further details on collection and processing. CrAg titers may be performed on stored specimens, in batch.

† Lab monitoring will consist of complete blood count (CBC) with platelets, Na^+, K^+, creatinine, ALT, and total-bilirubin.

9.2. CrAg Screening

CrAg screening may occur at time of CD4 testing with predominantly lab-based CrAg testing or by provider initiated testing. This CrAg screening is standard of care in accordance with national and WHO guidelines. This CrAg testing will occur at the CD4 referral laboratories for each site (Refer to Appendix C for site description). Point-of-care CrAg testing is also acceptable, if not previously tested (e.g. insufficient specimen for CrAg testing, discarded specimen)

when performed by a trained research staff member, as per the Delegation of Responsibilities log. A positive CrAg result is generally considered a critical lab value. Upon notification of the CrAg result, clinic staff and/or research study staff will attempt to contact the person to have them return to clinic on an urgent basis. Contact may be by telephone, alternative listed contact in their clinic medical record, or active patient tracking.

If leftover specimens exist, it is acceptable to test for ALT and pregnancy using this specimen.

9.3. Pre-Entry Visit

Pre-Entry Visit will assess eligibility for study enrollment by research staff. The research staff will perform the symptom-screen for meningitis, to confirm that the patient has no signs or symptoms concerning for meningitis. This is based on the healthcare provider's clinical impression.

If the patient is otherwise asymptomatic, and there are no contraindications to study participation, the patient will be asked to provide informed consent for possible study participation (if eligible). HIV care (prescription of ART and fluconazole) from this point on will be performed by research staff until completion of 24 weeks of the study, or if the participant withdraws consent for further participation. Informed consent will be obtained by study personnel. Women of reproductive potential **must** have a pregnancy test performed on either blood or urine. This may be performed as point of care testing or at the local site laboratory. If they are pregnant at time of enrollment, they are not eligible for the study.

Activities shall include:

- Clinical Assessment for Symptomatic Meningitis
- Informed Consent
- Pertinent Medical History
- Checklist of Inclusion/Exclusion Criteria
- Pregnancy Testing (if not already obtained on CRAG screening sample)
- ALT Testing (if not already obtained within 30 days of pre-entry visit)
- EKG with QTc measurement.
    - QTc must be <450ms for participation.

If the patient has signs or symptoms concerning for symptomatic meningitis, as suggested by meningitis symptoms as per the clinician's judgment, the provider will refer the patient for further care, including consideration of a lumbar puncture if thought to be necessary. Referral will be made to the appropriate healthcare facility, which can perform a diagnostic lumbar puncture. This is routine care and considered best clinical practice. Our study will adhere to this routine standard of care practice. If 1) lumbar puncture does not suggest features of meningitis, 2) CSF is CrAg-negative, and 3) there is no longer a suspicion for meningitis, the patient can be eligible, at provider discretion.

If not already prescribed by another healthcare provider, fluconazole should be prescribed as soon as possible. CrAg+ persons may receive up to 7 days of fluconazole prior to randomization (at the enrollment visit) and still be eligible.

9.4. Enrollment Visit (Week 0)

If the participant is eligible for study participation, they will be randomized into the C-ASSERT trial. This may occur immediately after the Pre-entry visit (i.e. same clinic visit), or may require a second visit if all the inclusion/exclusion criteria results are not available (e.g. ALT lab result). Randomization is stratified by site. The day of randomization is Day Zero.

The following evaluations will be performed at the enrollment visit:

- Labs: CBC (with platelets), Na+, K+, Creatinine, ALT, and Total bilirubin;
- Patient Health Questionaire-9 (PHQ-9) questionnaire;
- Documenting concomitant medications;
- Research laboratory collections, as available at the sites, which may include: PaxGene RNA, DNA, PBMC, serum, and plasma – with subject written consent for specimen storage.

At enrollment, plasma specimens will be collected / stored for CrAg titer and pathogenesis investigations. A PAXGene RNA tube (2.5mL) may be collected per site with storage capacity depending on study site and available funding for storage and to perform further testing.

Study medicine will be dispensed by the study pharmacy. The initial escalation phase of dosing will be provided in a blister-pack with a numbered sequence of up to 30 days study medication.

Research staff will be responsible for refilling HIV medications and fluconazole for the duration of participation in the research study.

9.5. Follow-Up Detailed Study Visits

Follow up HIV care will occur with routine visits as per the standard of care. Specific detailed study visits will occur as per the Schedule of Events (Section 9.1). Detailed study visits will have completion of a visit CRF at weeks 2, 4, and every 4 weeks thereafter through study termination at 24 weeks. The study visit windows are $\pm$2 weeks. These detailed follow up visits shall document and assess for:

1) Symptoms consistent with cryptococcal disease and/or meningitis
2) Clinically significant serious adverse events
3) Medication adherence, problems, or discontinuation for study medicine, fluconazole, and ART
4) For women, last menstrual period while receiving $\geq$400mg/day fluconazole.

A checklist will be used to query for symptoms of serious adverse events, which shall include: meningitis, CNS symptoms, serotonin syndrome, acute angle closure glaucoma, bleeding episodes, and other episodes of clinical importance.

A mental health screen will be performed using Patient Health Questionaire-9 (PHQ-9) at enrollment, week 4, week 8, and week 12. After 16 weeks, the PHQ-9 will be performed as clinically indicated. The PHQ-9 has nine questions which assess for depression as well as suicidal ideations and self-harm. Refer to Manual of Operations (MOP) for more details on the PHQ-9.

In the event patients have transferred care, a telephone visit is acceptable for collection of data, per our intention to treat analysis. This is preferable to a missed visit and no data collection. The telephone visit will be used to assess for vital status (alive, dead) and for symptoms of meningitis. A telephone visit is not preferred but is preferable to no assessment.

9.5.1. Week 2 Visit: ART initiation

Pre-ART counseling and initiation of ART will occur per standard clinic protocols. ART initiation will be recommended at approximately 2-4 weeks after study entry. The exact timing of ART initiation will not be mandated by the protocol, as this will be performed per standard of care in clinics and at provider discretion. There is insufficient evidence to guide exact timing of ART initiation among patients with asymptomatic cryptococcal antigenemia. Similarly, participants with virologic failure will be recommended for switch to second line therapy at 2-4 weeks. At follow up visits, ART adherence will be assessed via participant interview. If there are concerns, pill counts and/or repeat ART counselling should be conducted.

9.5.2. Week 4 and 8 visits

A checklist will be used to query for symptoms of serious adverse events, which shall include: meningitis, CNS symptoms, serotonin syndrome, acute angle closure glaucoma (i.e. acute eye pain), bleeding episodes, and other episodes of clinical importance. Assessments will include:

- Symptoms consistent with cryptococcal disease and/or meningitis
- Clinically significant serious adverse events
- Medication adherence, problems, or discontinuation
- Patient Health Questionaire-9 (PHQ-9)
- Laboratory monitoring of: CBC (with platelets), Na+, K+, Creatinine, ALT, and Total bilirubin.
- EKG

In the event patients have transferred care, a telephone visit is acceptable for collection of data, per the intention to treat analysis. A telephone visit is not preferred but is preferable to no assessment.

At the week 4 visit, the initially prescribed blister pack of study medicine will be returned with pharmacy conducting a reconciliation of returned medications.

9.5.3. Week 12 Visit

A checklist will be used to query for symptoms of serious adverse events, which shall include: meningitis, CNS symptoms, serotonin syndrome, acute angle closure glaucoma (i.e. acute eye pain), bleeding episodes, and other episodes of clinical importance. Assessments will include:

- Symptoms consistent with cryptococcal disease and/or meningitis
- Clinically significant serious adverse events
- Medication adherence, problems, or discontinuation
    - Pill count of returned study medications.
- Patient Health Questionaire-9 (PHQ-9)
- Laboratory monitoring of: CBC (with platelets), Na+, K+, Creatinine, ALT, and Total bilirubin.
- EKG

At the week 12 visit, the participants shall be instructed to begin tapering by one study medicine tablet per week (3, 2, 1, and then stop). Study medications will be dispensed in a blister pack for the taper.

As the patient will be an outpatient, the exact taper will not be monitored. Please refer to Pharmacy SOP for further details about how study drug will be supplied and monitored.

9.5.4. Week 16 Visit

A checklist will be used to query for symptoms of serious adverse events, which shall include: meningitis, CNS symptoms, serotonin syndrome, acute angle closure glaucoma (i.e. acute eye pain), bleeding episodes, and other episodes of clinical importance. Assessments will include:

- Symptoms consistent with cryptococcal disease and/or meningitis
- Clinically significant serious adverse events

Any remaining study medicine should be returned at the week 16 study visit or termination visit, for destruction by the pharmacy. No remaining study medication is the expectation.

Laboratory monitoring shall be performed for follow up of any Grade $\geq$3 abnormality to document resolution. EKG monitoring shall be performed for follow up of any Grade >3 abnormality (i.e. QTc >500ms).

9.5.5. Week 20 Visit

A checklist will be used to query for symptoms of serious adverse events, which shall include: meningitis, CNS symptoms, serotonin syndrome, acute angle closure glaucoma (i.e. acute eye pain), bleeding episodes, and other episodes of clinical importance. Assessments will include:

- Symptoms consistent with cryptococcal disease and/or meningitis
- Clinically significant serious adverse events

For follow up of any unresolved Grade ≥3 abnormality, laboratory monitoring shall be performed to document resolution. EKG monitoring shall be performed for follow up of any unresolved Grade ≥3 abnormality (i.e. QTc >500ms).

9.5.6. Other Routine / Unscheduled Clinic Visits

At other routine clinic visits for HIV care being conducted for medication refills, if persons are asymptomatic, no CRF need be completed. If participants are ill, refer to Section 9.5.8 regarding sick visits.

9.5.7. Pregnancy Visit

At time of a concern for suspected pregnancy,

1) Pregnancy test shall be performed

If pregnant:

2) Site PI (or designee) must be notified within 24 hours.
3) CrAg titer on serum/plasma shall be performed.
4) Fluconazole dose reduction should potentially be dose reduced as considered per discussion between the site PI, clinical provider, and the participant. See discussion in section 11.5.
5) Sertraline/Placebo should be tapered as rapidly as is feasible, but with recognition that abrupt discontinuation can be associated with withdrawal may be elected to be continued or discontinued per discussion between the clinical provider and the participant. Sertraline is a FDA Category C, and SSRIs are generally thought to be a low risk. In general, study med tablets should be tapered approximately one per week. Refer to Section 11.5

9.5.8. Other Sick Visits

Study personnel shall evaluate participants who present for sick visits as clinically appropriate and refer for hospitalized care, as needed. All sick visits must have an assessment for serious AEs. The study shall provide an appropriate diagnostic evaluation for any new AE. Participants will not need to pay for this evaluation. Specimens shall be collected as clinically appropriate (e.g. CSF, serum, plasma, etc.) based on physician discretion of the illness present. Women with a concern for pregnancy should have a pregnancy test checked. A visit CRF shall be completed for sick visits, even if the ultimate reason for the visit does not qualify as a serious AE or Grade 3-5 AE.

9.5.9. Study Termination Visit

The final study visit shall be targeted at 24 ±2 weeks. The Termination CRF shall be completed. If the participant has transferred care, a telephone call is acceptable to document vital status and document any relevant serious adverse events (e.g. meningitis, hospitalization, etc.).

If persons are transiently lost to follow up, but return after 24 weeks, the Termination CRF shall be completed/updated with the known alive vital status. Similarly, if a participant is initially deemed as lost to follow up, but is later known to have died before 24 weeks, the CRF shall be updated.

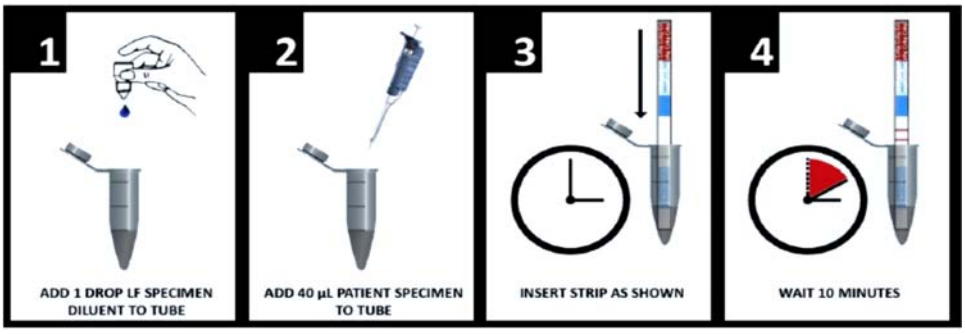
For persons lost to follow up, tracking is highly encouraged to assess their vital status and whether symptomatic meningitis has occurred. For deaths, the proximal contributing cause(s) of death shall be documented, if known. If a death occurs in a healthcare facility a post-mortem exam may be offered to the next-of-kin, as per routine medical practice.

For participants with ongoing clinical AEs (Grade 3-4) at time of 24 weeks, they should be continued to be followed until AE resolution and/or final outcome (Refer to Section 10.3 on AE reporting). Laboratory monitoring shall be performed for follow up of any Grade ≥3 abnormality to document resolution, as clinically relevant. EKG monitoring shall be performed for follow up of any unresolved Grade ≥3 abnormality (i.e. QTc >500ms).

9.6. Laboratory Evaluations

9.6.1. Cryptococcal Antigen lateral flow assay (CrAg LFA)

In July 2011, a lateral flow immunoassay was approved by the U.S. FDA for detection of cryptococcal antigen (Immy, Inc., Norman, Oklahoma). The CrAg lateral flow assay (LFA) is a rapid diagnostic dipstick immunochromatographic assay that provides a definitive result for presence of CrAg within 15 minutes. The procedure requires a drop (40µL) of serum, plasma, urine, or CSF specimen to be placed in a test tube, and the LFA dipstick is then placed into the same reservoir. If CrAg glucuronoxylomannan capsular polysaccharide is present in the specimen, once the lateral flow dipstick comes in contact with the specimen, the LFA will bind to the gold-conjugated, anti-cryptococcal antibodies on the test strip causing a visible line.



The LFA can be run individually using an Eppendorf tube (or similar) as a reservoir or can be batched on 96-well plates.

The CrAg LFA is an ideal point of care test, as persons with minimal training can perform it, without any additional laboratory equipment other than test tubes to hold the specimen. Only 1 drop of bodily fluid (~0.05 mL) is required to detect CrAg, and the CrAg LFA assay can be performed at room temperature, not requiring refrigeration or heat inactivation. Fingerstick CrAg testing is generally equivalent to serum or plasma CrAg testing.[45] CrAg titers will be performed by serial dilution at baseline using the screening or enrollment plasma specimen. Refer to lab SOP. CrAg titers may be performed on stored specimens.

9.6.2. Routine Laboratory Monitoring

Routine lab monitoring will be conducted at: Enrollment, 4, 8, and 12 weeks. This monitoring shall comprise of:

- Complete blood count (CBC) with platelets
- Serum electrolytes of Na^+, K^+
- Serum creatinine
- Serum hepatic tests of:
  - Alanine aminotransferase (ALT)
  - Total bilirubin (t-bili).

9.6.3. Additional Laboratory Monitoring

Persons with grade ≥3 lab abnormalities should continue to have monitoring conducted at a 2-4 week interval until the abnormality resolves to Grade 2 or less. For further details on clinical management, please see Manual of Operations.

Lab Test	Ref Range Ugandan Adult	DAIDS Grade ≥3 Threshold	Expected Event Rate
Hemoglobin, g/dL	M: 14.4 – 18.9 F: 10.9 – 16.7	M: <9 F: <8.5	50%
WBC x10^9/L	2.8 – 7.7	<1.5	5%
Platelet x10^9/L	M: 156 – 358 F: 125 – 445	<50	<5%
Sodium, mEq/L	138 – 150	<125 or >154	<2%
Potassium, mEq/L	M: 3.7 – 5.4 F:3.6 – 5.3	<2.5 or >6.5	<2%
Creatinine, mg/dL	M: 0.50 – 1.30 F: 0.50 – 1.20	M: >2.34 F: >2.26	<5%
ALT, U/L	M: 0 – 45 F: 0 – 35	M: >225 F: >175	<5%
T-bilirubin, mg/dL	M: 0.0 – 1.4 F: 0.0 – 1.3	>3.6 >3.4	<5%

9.6.4. Specimen Preparation, Handling and Shipping

All protocol specimens will be shipped using packaging that meets requirements specified by the International Air Transport Association Dangerous

Goods Regulations for UN 3373, Biological Substance, Category B, and Packing Instruction 650. Culture isolates, if obtained in this study, are to be shipped as specified for UN 2814 Category A Infectious Substances.

Biohazard Containment. Transmission of HIV and other blood borne pathogens can occur through contact with contaminated needles, blood, and blood products. Respiratory pathogens such *Mycobacterium tuberculosis* are transmitted by inhalation of droplet nuclei. Appropriate blood, secretion, and respiratory precautions will be employed by all personnel in the collection of clinical samples and the shipping and handling of all clinical samples and isolates for this study, as currently recommended by the Centers for Disease Control and Prevention in the United States, the WHO internationally, and the NIH.

9.7. EKG Monitoring

EKG monitoring will be performed with measurement of QTc interval at baseline, 4, 8, 12 weeks. Relevant drug dosing at these time will be:

Medicine	Enrollment	4 weeks	8 weeks	12 weeks	16 weeks
Fluconazole	0 *	400mg	400mg	400mg	200mg
Sertraline/Placebo	0	400mg	400mg	400mg	0 mg

* Persons may receive up to 7 days of antifungal therapy prior to trial enrollment.

The corrected QT interval (i.e. QTc) should be preferably calculated via either:

- Fridericia's method $QTc = \frac{QT}{\sqrt[3]{RR}}$, or (default QTc on GE Mac® 1200 EKG Instrument)
- Framingham method $QTc = QT + 0.154\ x\ (1 - RR)$

CrAg+ persons with a baseline EKG QTc ≥450 at the pre-entry visit are not eligible for trial enrollment, per the directive of the Funder. Per NIH rules, persons not eligible for trial participation may not have study personnel or study resources used to provide lifesaving treatment. The NIH does not support routine medical care, only research.

10. ASSESSMENT OF SAFETY

This is a clinical trial investigating whether adjunctive sertraline will lead to improved survival outcomes in HIV-infected persons with AIDS and cryptococcal antigenemia.

Fluconazole and sertraline have a long track record of safety with >30 million U.S. prescriptions of sertraline annually.

10.1. Safety Assessment Overview

Serious Adverse Event

A Serious AE is any adverse event, without regard to causality, that is or causes:

- life-threatening;
- death;
- hospitalization;
- persistent or significant disability or incapacity; or
- congenital anomaly or birth defect.

Any other medical event that, in the medical judgment of the Investigator, may jeopardize the subject or may require prompt medical or surgical intervention to prevent one of the outcomes listed above is also considered a Serious AE. A planned medical or surgical procedure is not, in itself, a Serious AE.

Routine outpatient medical therapy for a common, expected illness in the study population (e.g. malaria, TB) is not a Serious AE.

All AEs that do not meet any of the criteria for serious will be classified as *non-serious adverse events*.

Expected Adverse Events include:

- Death due to Cryptococcal infection;
  - *Comment: This is the primary endpoint and expected in up to 20-25% of participants.*
- Symptoms due to cryptococcosis and/or meningitis
- Hospitalization due to cryptococcosis (up to 30%)
  - *Comment: This is a Serious AE due to hospitalization, but is expected.*
- Common ART side effects (e.g. anemia)
- Immune reconstitution inflammatory syndrome
- Tuberculosis (5-10%)
  - *Comment: If hospitalization is required, this is a Serious AE.*
- AIDS-related opportunistic infections.
- ART-related liver injury (4%)[89]

These expected events will be reported to the IRB in aggregate via interim reviews. The goal of aggregate reporting is to continue to assure subject safety and to perform reporting in a meaningful manner which individual reporting cannot provide. The DSMB will monitor the primary endpoint of cryptococcal meningitis + death for overall safety/efficacy.

Unexpected Adverse Events

All unexpected serious AEs will be reported, regardless of causality to sertraline to the DAIDS medical officer. Examples would include severe hepatotoxicity while taking fluconazole and sertraline (without ART), which would be unexpected. Clinically significant serotonin syndrome is unexpected. Unexpected AEs will be adjudicated by the Trial Safety Committee (Section 12.7.4) and if deemed to be potentially-related to sertraline, reported to the FDA (Section 10.6).

10.2. Grading Adverse Event Severity

Grading clinical severity of AEs will occur via the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (version Nov 2014).

- Grade 3: Severe – "Severe symptoms causing inability to perform usual social & functional activities with hospitalization indicated."
    - Any AE which results in necessary hospitalization is at least Grade 3
    - Any AE of sufficient severity which prompts immediate study medicine discontinuation is at least a Grade 3 event.
        - An AE resulting in a dose decrease or early taper is not necessarily a Grade 3, unless it meets Grade 3 criteria.
- Grade 4: Potentially Life-Threatening – "Potentially life-threatening symptoms causing inability to perform basic self-care functions with urgent intervention indicated to prevent permanent impairment, persistent disability, or death."
    - Standard treatment for illness, which if not treated is potentially life threatening (e.g. malaria, TB), is not considered Grade 4 AE unless the present symptoms are potentially life threatening. For example, uncomplicated malaria is not a Grade 4 AE but cerebral malaria is a Grade 4 AE. Severe malaria requiring hospitalization is at least a Grade 3 AE.
- Grade 5: Death

10.3. Recording of Serious AEs

All serious AEs occurring from study entry through study termination will be recorded.

- Non-fatal AEs will be recorded on the 'AE CRF.'
- Fatal AEs, dependent on the time course of the death, *may* be recorded on an 'AE CRF,' but *must* be recorded on the 'Termination CRF.'

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 6-month reporting period must be followed up to determine the final outcome. All unresolved serious AEs at 6-months should be followed until the events are stabilized or otherwise explained.

Cryptococcal-related deaths and incident cryptococcal meningitis will not be included in the analysis of AEs, but these will be analyzed as separate endpoints. Cryptococcal-free survival is the primary endpoint of the trial.

When selecting a primary AE term to report, sites should select the term that best describes what occurred to the participant. For example, a participant may present with itching, urticaria, flushing, angioedema of the face, and dyspnea. If the underlying diagnosis is determined to be an acute allergic reaction, sites should report "Acute Allergic Reaction" as the primary AE term.

10.3.1. Laboratory AEs

Laboratory abnormalities do not need to be individually reported on an 'AE CRF' unless the event results in a Serious AE. All laboratory results shall be reported on 'Blood Results CRF.' Lab abnormalities will be summarized from the statistical database and graded for severity according the DAIDS Toxicity Table version Nov 2014. These laboratory AEs will be summarized separately from clinical AEs.

10.4. Adverse Event Procedures and Reporting Requirements

To comply with national regulations, reports of all ***serious unexpected*** adverse events, related or potentially related to the study medication will be submitted to the IRB of record and sponsor within 3 working days of the study site awareness of the AE. Reporting will be via the 'Adverse Event Reporting CRF.' Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's binder.

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

- Study identifier (C-ASSERT)
- Study Site
- Medical record number
- Date of event
- A description of the event
- Medical treatments given / stopped
- Working diagnosis
- Current vital status

Within the 7 working days following the event, the investigator will provide further information on the AE Reporting 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 DAIDS and IRB of record.

10.5. Expedited AE Reporting Requirements for the Study to NIH

The Suspected Unexpected Serious Adverse Reaction (SUSAR) Reporting Category, as defined in Version 2.0 of the DAIDS Expedited AE Manual, will be used for this study. The study products for which expedited reporting are required are: sertraline/ placebo. Unblinding will not routinely occur for event reporting, unless as requested by the IRB, FDA, or Sponsor.

The Adverse Events are not reported under the DAIDS SUSAR system, which must be reported to the DAIDS medical officer are:

- Serious AEs; AND
- Unexpected; AND

- Related or Potentially related to Sertraline

Individual reporting of each SAE is not recommended as the expected event rate is 30-35% with the standard of care therapy. The primary endpoint is a composite endpoint of death and/or cryptococcal meningitis (which requires hospitalization which is an SAE). The DSMB is monitoring this primary endpoint for subject safety. Individual reporting of primary endpoint events in a blinded trial provides no additional safety for subjects.

Unexpected AEs or SAEs with potential relationship to sertraline would be important for safety monitoring.

A *Trial Safety Committee* will review each unexpected SAE and perform an early independent review of trial safety (mortality + SAE incidence) after 75 subjects have been enrolled and accrued 3 months follow up. Refer to Section 12.7.4.

10.6. FDA IND and NDA Safety Reporting

For unexpected SAEs, reporting to FDA and NDA will be as follows:

"FDA believes that the sponsor is better positioned than the individual investigator to assess the overall safety of the investigational drug because the sponsor has access to serious adverse event reports from multiple study sites and multiple studies and is able to aggregate and analyze these reports. Moreover, the sponsor is more familiar with the drug's mechanism of action, class effects, and other information. For these reasons, investigators must immediately report any serious adverse event to the sponsor, whether or not the investigator considers the event to be drug related (21 CFR 312.64(b))."

According to 21 CFR 312.32(c)(4), a sponsor of a clinical study of a drug marketed or approved in the United States that is conducted under an IND must submit IND safety reports for unexpected suspected adverse reactions that are observed in the study

Unanticipated / unexpected SAEs will be individually reported using FDA Form 3500A as a '15-day IND safety report' or '7-day IND safety report.'

- **15 days**: "The time frame for submitting an IND safety report to FDA and all participating investigators is no later than 15 calendar days after the sponsor determines that the suspected adverse reaction or other information qualifies for reporting (21 CFR 312.32©(1))."
- **7 days**: "The requirement for reporting any unexpected fatal or life-threatening suspected adverse reaction to FDA is no later than 7 calendar days after the sponsor's initial receipt of the information (21 CFR 312.32©(2))."

This can be sent to FDA as an Electronic Common Technical Document (eCTD) format (e.g. PDF) via the FDA Electronic Submission Gateway.

Annual Reporting of Aggregate AEs will occur with annual FDA IND reports, in addition to expedited unexpected SAE reporting, based on the adjunction of the Trial Safety Committee (Section 12.4.7).

All reports submitted to the FDA will also be submitted to the Uganda NDA.

10.7 Safety Reporting Summary

The summary of Adverse Event reporting and timeline is as follows.

Grade	Serious	Unexpected	To whom to Report	What is reported?	Timeline †
1-2	No	Yes / No	None	None	N/A
3-4	No	No	None	*Clinical AE* CRF	DSMB & Annual IRB Reporting
1-3	No	Yes	None	*Clinical AE* CRF if Grade ≥3	DSMB & Annual IRB Reporting
Any	Yes	No	DAIDS MO	*Clinical AE* CRF or *Termination* CRF	Trial Safety Committee review after each 40 subjects have accrued 3 months
Any	Yes	Yes	IRB	*Clinical AE* CRF	≤ 3 working days
			FDA, NDA	FDA Form 3500A	≤ 7 calendar days
			DAIDS MO	*Clinical AE* CRF	≤ 3 working days
Lab Value*	No	No	None	*Blood Results* CRF	DSMB & Annual IRB Reporting

*Lab abnormalities not requiring clinical intervention to not need to be individually reported on the *Clinical AE* CRF. These will be summarized by DAIDS toxicity via the trial's statistical database for IRB reporting and DSMB monitoring. Lab abnormality AEs necessitating clinical intervention require reporting as a Clinical AE and reporting as above.

† Additionally, monthly reporting on AE incidence will occur with progress reports to NIH NIAID DAIDS medical officer (Refer to Section 12.7.2). All AEs will be summarized for annual reporting to IRBs, DSMB, U.S. FDA, and Uganda NDA.

11. CLINICAL MANAGEMENT

11.1. Clinical Management of Adverse Events

Unanticipated and anticipated toxicities will be graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2014. AEs assessed as related to non-study drugs (concomitant medicines) should be handled according to the relevant package inserts and the best medical judgment of the site investigator.

11.2. Serotonin Syndrome

Serotonin syndrome does not generally occur with SSRIs alone. Even in overdose, the incidence of serotonin syndrome with sertraline is 6% when the

Physical presentation of serotonin syndrome includes:

- Confusion, agitation, reduced level of consciousness, seizures,
- **Clonus/myoclonus**, **hyperreflexia**, tremors, muscle rigidity,
- **Ocular clonus** (e.g. -slow, continuous, horizontal eye movements)
- Ataxia (lack of voluntary coordination), akathisia (e.g. restlessness/constant motion),
- Hyperthermia (>38ºC),
- Hypertension, tachycardia,
- Diaphoresis, lacrimation, mydriasis, shivering, and/or diarrhea.

Hunter Criteria for Serotonin Syndrome - a patient must have taken a serotonergic agent and meet **ONE** of the following conditions:

- Spontaneous clonus;
- Inducible clonus PLUS agitation or diaphoresis;
- Ocular clonus PLUS agitation or diaphoresis;
- Tremor PLUS hyperreflexia;
- Hypertonia PLUS temperature above 38ºC PLUS ocular clonus or inducible clonus.

Clonus (spontaneous, inducible and ocular) is the most important sign in the Hunter Serotonin Toxicity Criteria. Clonus is rare in cryptococcosis. Serotonin syndrome is a diagnosis of exclusion. Metabolic, toxic, and infectious causes should be ruled out based on the patient's history and presentation. Differential diagnoses are:

- Meningitis/encephalitis (headache uncommon with serotonin syndrome, clonus not present in cryptococcal meningitis)
- Delirium tremens from alcohol withdrawal (assess by history),
- Malignant hyperthermia or intoxications with adrenergic or anticholinergic meds (assess by concomitant medication history).

Medical Management of Serotonin Syndrome

- Discontinuation of all serotonergic agents.
  - Review concomitant medicines. SSRIs alone do not cause serotonin syndrome but can be caused via interactions with other prohibited medicines such as MAO inhibitors, pimozide, and serotonergic drugs:

sumatriptan, zolmitriptan, tricyclic antidepressants, fentanyl, lithium, tramadol, tryptophan, buspirone, and St. John's Wort.

- Supportive care aimed at normalization of vital signs
  - Intravenous fluids
  - Oxygen if hypoxia present
- Sedation with benzodiazepines

The Site Principal Investigator and C-ASSERT Principal investigator(s) must be contacted upon site awareness of a possible serotonin syndrome event.

Participants with severe serotonin syndrome (involving altered mental status) require hospitalization and must have their Sertraline/Placebo held for 24 hours, concomitant medicines evaluated, and have a repeat clinical evaluation within 1 day. This is an unexpected Serious Adverse Event. Serotonin syndrome symptoms should generally dissipate in 24 hours. Worsening symptoms beyond 24 hours should prompt consideration of alternative diagnoses, including lumbar puncture for CSF analysis.

For persons with mild serotonin syndrome (e.g. without mental status changes), holding medication for 48 hours, and dose reduction by ≥25% is appropriate.

11.3. Hepatic Dysfunction

ALT >5x upper limit of normal is exclusion criteria for enrollment. Participants who develop hepatic dysfunction while on study drug should potentially have study medicine dose reduced and more frequent checking of liver function tests (see below). The incidence of hepatotoxicity among persons taking sertraline is <1 in 1000 (FDA Package Insert). Hepatotoxicity due to antiretroviral therapy is 2-18%, dependent on the population.[89] Based on historical experience in Uganda among persons with HIV/AIDS, the expectation is an approximately 4-6% incidence of liver function test abnormalities after starting ART, related to the immune reconstitution towards liver pathogens (e.g. hepatitis B).

Liver Function Test Abnormality	Study Medicine Action	Next Lab Recheck
ALT <2.5x ULN	Continue	As per protocol
ALT 2.5-5x ULN	Continue	4 weeks
ALT >5x ULN	Dose reduce by ~25%	≤2 weeks
Bilirubin <2.5x ULN	Continue	As per protocol
Bilirubin 2.6-5x ULN	Continue	4 weeks
Bilirubin 2.6-5x ULN & ALT 2.5-5x ULN	Dose reduce by ~25%	≤ 2 weeks
Bilirubin >5x ULN	Dose reduce by ~25%	≤ 2 weeks

11.4. EKG QTc Abnormalities

Clinical management actions for prolonged QTc are based on FDA E14 guidance for Safety Monitoring and Discontinuation Criteria (2.1.2).[64]

DAIDS Toxicity Grade	QTc Threshold	Study Drug Action	Repeat EKG *
1	450-470 ms	Continue without interruption, at the discretion of the site investigator.	≤ 4 weeks
2	471-500 ms	Continue without interruption, at the discretion of the site investigator.	≤ 2 weeks
3	>500 ms	Reduce study drug dose by at least 25% and have an EKG repeated within ≤2 weeks.	≤ 2 weeks
4	Torsade de pointes, etc.	Discontinue fluconazole and sertraline study drug immediately.	≤ 1 day

* Repeat EKGs will be performed beyond the routine duration of EKGs. A QTc change >60ms which remains within normal QTc range <450ms will not prompt additional action.

After a dose reduction, based on repeat EKG QTc, investigators may resume the prior study drug dose at their discretion. Sertraline does not typically have an EKG QTc effect; however, other concomitant medications may cause QTc effects.

11.5. Pregnancy

Women who become pregnant while receiving fluconazole must have:

A) **CrAg titer** performed to gauge the current burden of cryptococcal infection.

B) **CD4 count** measured.

C) **PI or designee must be contacted** within 24 hours of site awareness of a pregnancy (preferably immediately).

D) **Study Medicine Tapered** at 1 tablet per week.

E) **Referral for prenatal care**, outside of the study

Based on these results and in consultation with the site PI, consideration of altering fluconazole dosing should be one of the following:

1) Dose reduction to ≤200 mg/day or

2) Discontinuation if they have received >8 weeks of therapy.

3) Discontinuation if their CrAg Titer ≤1:80 AND CD4 >50 cells/μL.

In August 2011, high dose fluconazole (400-800 mg/day) was labeled as FDA pregnancy category D, meaning that there is positive evidence of human fetal risk based on human data. In humans, there are a total of nine case reports that suggest that dose-dependent teratogenicity during the first trimester of pregnancy may be associated with a rare and distinct set of birth defects in infants.[90-97] These skeletal defects are similar to those described in animal studies. Adverse events have been observed in some animal reproduction studies. When used in high doses, fluconazole is teratogenic in animal studies. FDA stated guidance is:

"Use of long-term, high-dose (400-800 mg/day) fluconazole during the first three months of pregnancy (first trimester) may be associated with a rare and distinct set of birth defects in infants."

High dose fluconazole is potentially teratogenic in the first trimester of pregnancy. This risk must be weighed against the risk of maternal death due to cryptococcal meningitis. Most azole antifungals, including fluconazole, are recommended to be avoided during pregnancy.

Conversely, CrAg+ persons with CD4<100 who do not receive antifungal therapy have 100% mortality in two studies of CrAg+ persons,[3, 12] so the risk vs. benefit of removing life-saving therapy to avoid a potential teratogenic effect is complicated and best discussed with the PI(s) with customized therapy plan.

Lower doses (150 mg as a single dose) do not suggest an increase risk to the fetus.

If the participant continues her pregnancy, the site or patient are encouraged to prospectively register her pregnancy in the "Antiretroviral Pregnancy Registry" (In US and Canada: 1-800-258-4263, international: +1-910-256-0238).

Table 7. Five case reports of fluconazole-related teratogenicity

Case (Ref)	Indication	Dose of fluconazole	Weeks	Timing in pregnancy	Outcome
Case 1 [90]	Coccidioidal meningitis	400mg daily	27	Weeks 0-27	Mother: premature rupture of membranes at 27 weeks. Infant: Multiple skeletal abnormalities; facial deformities including cleft palate. Died after birth.
Case 2 [91]	Coccidioidal meningitis	800mg daily	37	Weeks 0-7 Weeks 9-38	Infant: Skeletal, facial, cardiac abnormalities
Case 3 [91]	Coccidioidal meningitis	400mg daily	16	Weeks 0-16	Infant: Skeletal abnormalities, cleft palate, pulmonary and cardiac complications. Died at 3 months.
Case 4 [92]	Coccidioidal meningitis	400mg daily 800mg daily 1200mg daily	19	Weeks 0 - 4 Weeks 5 - 9 Weeks 22-31	Mother: Premature rupture of membranes at 31 weeks. Infant: Skeletal & facial abnormalities
Case 5 [93]	HIV and vaginal candidiasis	800mg daily	34	Weeks 0-23 Weeks 27-37	Infant: Skeletal abnormalities, abnormal facies
Case 6 [94]	*Candida parapsilosis* fungemia	200mg daily	N/A	Unspecified (1st trimester start)	Infant: No abnormalities
Case 7 [95]	*Candida albicans* fungemia	400mg daily	4	Weeks 10-14	Not specified
Case 8 [96]	*Candida albicans* fungemia	400mg daily	7	Weeks 16-23	Mother: No abnormalities Infant: No abnormalities
Case 9 [97]	*Candida glabrata* fungemia	600mg daily	3	Weeks 14-17	Infant: No abnormalities

11.6. Failure of Preemptive Treatment

Persons with failure of preemptive antifungal therapy who develop symptomatic cryptococcal meningitis must be referred to the local/regional hospital for meningitis management. The study should facilitate this transportation. Participants will otherwise remain in the study, unless consent is withdrawn.

11.7. Criteria for Discontinuation of Trial Participation

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

- 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 their HIV care, 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 they withdraw from the study. The participants will be offered HIV medicines and fluconazole, per Ugandan standard of care. If the patient is on high dose sertraline ($\geq$200mg daily), the medication will be tapered slowly to avoid adverse side effects.

11.7.1. Criteria for Premature Study Medicine Discontinuation

A study subject shall discontinue the blinded study medicine if:

- 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.
- Withdraw of informed consent with a request by participant to withdraw.
- Pregnant women should have their study medicine rapidly tapered by 1 tablet per week and dose of fluconazole reduced to $\leq$200mg/day.

Participants may elect to discontinue the study medicine and remain in the trial, per the intention to treat analysis. Vital status and signs/symptoms of meningitis will be obtained at 24 weeks post-enrollment. If a participant is voluntarily terminating antifungal therapy before 8 weeks, the site PI must be notified immediately, as the result may be ~100% mortality.

11.7.2. Criteria for Study Medicine Dose Reduction/Resumption

The study medicine/placebo may be dose reduced for participant safety in the event of an AE which in the opinion of the investigator is temporarily and causally associated with sertraline. A prime example would be serotonin syndrome with clear causality. Other toxicities with possibility causality shall have management based on the best judgment of the clinical investigator.

Dose reduction should be at least 25% (i.e. from 4 to 3 tablets/day) but may be a higher dose reduction based on the timing and severity of the toxicity. Once the toxicity has resolved, investigators may resume the prior dose (i.e. 4 tablets) at

their discretion. If the toxicity recurs, sertraline should not be further dose escalated. In general, drug-related toxicities are more likely to be caused from other concomitant medicines, such as ART; however, investigator judgement is paramount.

12. STATISTICAL CONSIDERATIONS

12.1. Study Endpoints

All participants (Phase II and Phase III) will contribute data for the primary and secondary endpoints.

12.1.1. Primary Endpoint

1) 6-month cryptococcal meningitis free survival with retention-in-care
   - Those who die of any cause are failures
   - Those developing symptomatic cryptococcal meningitis are failures
   - Those lost to follow up and unable to be tracked are considered failures

12.1.2. Secondary Endpoints

2) 6-month survival time
3) Incidence of symptomatic cryptococcal meningoencephalitis
4) Incidence of Clinical Adverse Events (Grade 3,4,5)
5) Incidence of Laboratory Grade 3-5 Adverse Events
6) Incidence of premature study drug/placebo discontinuation
7) Prevalence of Depression by PHQ-9 scale over time.

12.1.3. Exploratory Endpoints

8) Medication adherence by blood levels
9) Risk factors for cryptococcal-free treatment failure
10) Adherence with the cascade of HIV and antifungal care (e.g. time to: return to clinic, antifungal therapy, ART initiation)
11) Use of text message or other reminders to minimize loss to follow up

12.2. Analysis Plan

We will compare survival by time-to-event analysis for cryptococcal-free survival using Log-rank test and survival displayed with a Kaplan-Meier survival curve with an indicator variable for treatment assignment. Analysis will be by intention-to-treat.

All asymptomatic CrAg+ persons will be included (i.e. denominator). Persons symptomatic at baseline (i.e. pre-existing meningitis) would not be enrolled but referred to hospital for cryptococcal meningitis care. The CrAg-negative 6-month survival rate among persons with CD4<100 and ART is ~89% in Uganda (e.g. **Figure 3**), thus if cryptococcal infection is fully negated, approximately 89% 6-month survival would be the target survival.

12.3. Analysis Plan Secondary and Other Endpoints

- **6-month Survival Time**
  Survival time will be analyzed using Log-rank test and displayed via Kaplan-Meier survival curve. Persons lost to follow up will be considered failures. All participants will be censored at 6 months.
- **Incidence of Symptomatic Cryptococcal Meningoencephalitis**

The cumulative incidence function will compare the randomized groups for incidence of symptomatic cryptococcal-related meningitis/encephalitis, which will account for the competing risk of death.[31]

Symptomatic meningoencephalitis will be defined as meningitis symptoms plus:

1) *Cryptococcus* culture positive meningitis,
2) CSF CrAg+, or
3) Cryptococcoma(s) by neuroimaging or post mortem exam.[98]

- **Incidence of Adverse Events**

  The cumulative incidence function will compare the randomized groups for incidence of Grade 3, 4, 5, and Serious AEs, which will account for the competing risk of death.[31]

  Cryptococcal-related deaths and incident cryptococcal meningitis will not be included in the analysis of AEs, but these will be analyzed as separate endpoints (as above).

- **Incidence of Laboratory Adverse Events**

  The cumulative incidence function will compare the randomized groups for incidence of laboratory adverse events of Grade 3, 4, 5, which will account for the competing risk of death.[31] A laboratory value which results in clinical action meeting the definition of a Serious AEs (e.g. hospitalization), is a Serious AE and will be also included above. Asymptomatic / minimally symptomatic lab abnormalities which do not result in hospitalization will be summarized herein. Based on the lab monitoring, this includes hematologic, elctrolyte, renal, and liver lab testing labnormalities.

  The frequency of EKG QTc abnormalities by Grade will be separately reported and do not need to be reported on the Clinical AE CRF unless symptomatic.

- **Incidence of Premature Study Drug/Placebo Discontinuation**
  The cumulative incidence function will compare the randomized groups for all-cause incidence of study drug/placebo discontinuation.[31] Premature discontinuation of the study medicine/placebo may occur from withdrawal of consent, intolerability, non-adherencce, adverse event, or other potential reasons.

- **Prevalence of Depression**

  Prevalence of Depression as per PHQ-9 questionnaire will be collected at baseline, 4 weeks, 8 weeks, and 12 weeks routinely. A mixed effects regression model with a random intercept for individual will be used to account for the intra-subject correlation induced by repeated PHQ-9 measures over time. An interaction indicator variable of time and treatment groups will assess differences in PHQ-9 depression scores. Additionally, we will summarize the prevalence of depression (by severity) from baseline through 12 weeks by randomized group.PHQ-9 Total Score Depression Severity Scoring:

  1-4 Minimal depression
  5-9 Mild depression
  10-14 Moderate depression
  15-19 Moderate severe depression

20-27 Severe depression

- **Adherence**

Adherence: We will perform a 1:1 case: control study measuring fluconazole and sertraline plasma levels in those with treatment failure vs. controls to objectively assess non-adherence (i.e. absence of sertraline) or malabsorption or partial adherence (i.e. lower levels). Those with treatment failure are those who develop cryptococcal meningitis and/or death during the 6 month follow up period. Controls will be randomly selected amongst the group who achieved cryptococcal-free survival at 6 months. Controls will be matched to cases by clinic site and time of enrollment (+/- 6 months). If non-adherence is common in treatment failure, then improved patient education should be part of an enhanced package of care for the future. Plasma fluconazole and sertraline levels will be measured retrospectively at the University of Minnesota Clinical Pharmacology Analytical Services Laboratory, which has validated assays for both compounds.[33]

- **Biomarker Analysis**

First, we plan to conduct a nested case-control as a sub-study to measure plasma biomarkers on CrAg+ persons at screening and again at 4 weeks versus controls. Cases will be those with the primary outcome status of cryptococcal meningitis or death. Controls will be CrAg+ survivors who do not develop cryptococcal meningitis through 24 weeks. Controls will be randomly selected with matching based on site. We will test both routine clinical markers (e.g. CRP, d-dimer). As we have reported in two prior HIV cohorts with AIDS that elevated pre-ART plasma CRP is associated with risk of unmasking IRIS events and/or death.[99, 100] Specifically, in a cryptococcal meningitis survivor cohort, pre-ART serum CRP >32 mg/L was associated with 8-fold higher risk of death on ART.[100] In a separate HIV cohort with CD4<200 cells/μL, every two-fold increase in pre-ART plasma CRP was associated with a 2.1-fold increased odds of IRIS and 2.1-fold increase in odds of new AIDS event or death.

Second, we plan to measure ~17 plasma cytokines / chemokines via Luminex multiplex assay (Bio-Rad, Hercules, CA) in order to generate an immune signature associated with future meningitis and/or death. Specimens will be stored with measurements in batch. Analysis will compare mean levels of $\log_2$ transformed biomarkers compare cases (meningitis or death) vs. controls (survivors) via logistic regression with and without adjustment for demographic or other variables (e.g. CrAg titer) associated with treatment failure.

Third, we will compare cell phenotype and activation using flow cytometry among a subset of cases and controls to determine the cellular phenotype and activation pattern that are associated with development of cryptococcal meningitis and/or death.

We *hypothesize* CrAg titer $\geq$1:160 and plasma CRP will also be predictive herein for the Aim 2 analysis of CrAg+ persons determining increased risk of failure of preemptive therapy (i.e. cryptococcal meningitis or death). The biomarker analyses are primarily exploratory, but with hypotheses similar to the published experience with cryptococcal meningitis.[99, 100]

Lastly, we will collect RNA from whole blood in PAXGene tubes at selected sites to potentially perform a future exploratory case:control transcriptome analysis of the gene signature associated with cryptococcal disease progression and/or death.

12.4. Sample Size Considerations

To enroll 600 people in total (Phase II and Phase III), with a CrAg prevalence rate of approximately 5%, we anticipate screening ~12,000 persons with a CD4<100 cells/μL.

With 300 subjects enrolled per group (n=600 in total), a two-sided log-rank test achieves 80% power at a 0.05 significance level to detect a hazard ratio of 0.60 when the proportion with cryptococcal-free survival with retention-in-care in the control group is 75%, equating to an approximate survival of 84% in the intervention group.[101] There is 90% power to detect a hazard ratio of 0.55 equating to 85% cryptococcal-free survival.

Approximately 120 events are expected during the course of the study. For the biomarker case/control study if there are 120 participants per group then we can detect a 0.36 standard deviation difference in a biomarker level between cases and controls with 80% power and a 0.05 significance level. Similarly, if there are 100 participants per group we can detect a 0.40 standard deviation difference, and with 80 participants per group we can detect a 0.44 standard deviation difference.

The number of specimens to be measured for biomarker analyses will depend on the event rate and financial resources available. Ideally, at least a 1:1 case:control study would be performed comparing all cases of death or meningitis vs. equal number of randomly selected controls of the survivors without meningitis through 24 weeks. A 1:2 case:control study would be better, or testing the entire cohort would be most ideal. The number of specimens tested will depend on the financial resources available.

12.5. Randomization / Stratification

We will use a permutated block randomization in a 1:1 allocation (n=300 per arm). Randomization will be stratified by district (e.g. Kampala, Mbarara, Masaka). Randomization codes will be developed and held by the trial biostatistician at the University of Minnesota.

Randomization schedules will be supplied to the pharmacy at each clinical trial 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 biostatistician will have access to both the participant study identification number and the randomization group. Detailed instructions for study drug processing, labeling and dispensing are included in the Pharmacy SOP.

12.6. Blinding Procedures/ Unblinding Procedures

This is a double-blind, placebo controlled study. Study medicine and placebo will be blinded and matched. Sertraline is a known medicine with an extensive safety record. In the event of Serious AE, the study medicine may be discontinued at the providers' decision. Due to the safety record of sertraline, unblinding will not occur for the participant or investigators. The treatment for a serious AE related to the study medicine is discontinuation of the medicine. Unblinding would not change clinical management and has the potential to introduce unintended bias. The data and safety monitoring board will be unblinded to serious AEs.

Participants may be switched to open label sertraline at clinician discretion if they are diagnosed with severe depression after week 12. For persons with perceived intolerance or toxicity, dose reduction may occur at provider discretion.

12.7. Data and Safety Monitoring

*Safety oversight***.** Safety oversight will be under the direction of an independent data and safety monitoring board (DSMB). A standing NIH NIAID DSMB will have oversight with use the: "Charter for the Data and Safety Monitoring Boards of DAIDS, NIAID." The most current version (presently 24 June 2015) is available at: https://www.niaid.nih.gov/sites/default/files/dsmb_charter.pdf

The NIAID Complications and Co-infections (CC) DSMB will have oversight responsibilities. The executive secretary for NIAID-CC-DSMB contact is:

Sally Hunsberger, PhD
Mathematical Statistician
5601 Fishers Lane, rm 4D13
Rockville MD, 20892
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The NIAID CC DSMB is scheduled to conduct an initial protocol review on September 6, 2017. Thereafter, the DSMB will conduct reviews after every 6-12 months of enrollment and will meet at least annually. Based on the accumulating C-ASSERT trial data, the DSMB may meet more frequently. A Lan-DeMets spending function analog of the O'Brien-Fleming boundaries will guide early stoppage. A formal sample size re-estimation will occur at time of the second DSMB report to be considered by the DSMB. This sample size estimation will assess the baseline assumptions of 75% incidence of cryptococcal free survival with retention in care versus the current observed experience to date.

DSMB Reports will include the information contained in the Progress Reports and Clinical Quality Management Plan reports as well as an analysis for the primary endpoint.

Open DSMB reports will be prepared with pooled demographic and trial progress data. This open report will be blinded to randomization allocation.

Closed DSMB reports will have data prepared by randomization group for demographics of enrolled participants and the primary outcome.

Baseline demographic features of each study arm will be summarized, with statistical testing as appropriate for nominal and continuous variables to assure adequacy of randomization. Any parameters found to be potentially different between randomization groups (P<0.1) and associated with outcome may be included in a multivariable adjusted Cox regression analysis.

12.7.1. Planned Interim Analyses and Stopping Guidelines

DSMB will monitor the study for safety endpoints, accrual issues, and general implementation of the study beginning approximately 6 months after trial initiation.

The first DSMB interim analysis will be prepared after approximately 100-150 subjects are enrolled and have endpoint data, and the DSMB will review accruing data *at least* annually thereafter. The exact timing is based on the DSMB schedule. An early DSMB review may be called for by the Trial Safety Committee if there are initial safety concerns which occur in the Phase II trial. DSMB members can also request additional more frequent reviews.

A Lan-DeMets spending function analog of the O'Brien-Fleming boundaries will be provided at each DSMB report for the stopping guidelines for 6-month cryptococcal-free survival outcome.

The O'Brien-Fleming boundaries will be truncated at alpha=0.002 (|Z|>3.09). The provided **Table 8** assumes three interim analyses and a final analysis with an overall two-sided cumulative alpha=0.05.

Interim Analysis	Sample Size	| Z |	P-value	Cumulative Alpha
1	~150 (25%)	> 3.09	0.002	0.0020
2	~300 (50%)	> 3.09	0.002	0.0037
3	~450 (75%)	> 2.38	0.016	0.0193
Final	~600 (100%)	> 2.02	0.031	0.0500

Sample Size Re-estimation: For the second DSMB review, when approximately 50% of the participants have been enrolled, a formal sample size re-estimation will occur to assess whether the initial assumptions for the primary endpoint (25% in the control group) were correct. The sample size re-estimation will be based on the pooled (and blinded) event rate and the 9% absolute difference in mortality expected between the groups. The sample size re-estimation will not take into account the interim treatment effect. Based on the results of the sample size re-estimation the DSMB may recommend that the study continue as planned or to increase the sample size, if the event rate is lower than a priori expectations. The investigators will not receive this report.

Futility Analysis: The expected accrual rate is 125-150 participants per year. Enrollment in year 1 will likely be lower due to staggered starting of clinical sites, each of which is dependent on Funder permission to initiate. At the second DSMB the accrual is less than 75% of what is expected, the DSMB committee may ask the study team to submit a formal plan to increase enrollment. If the conditional power is <20% after the third interim analysis (or when approximately 75% of participants have been enrolled) or thereafter, trial discontinuation may be

considered by the DSMB. The DSMB will be given conditional power under both the design and the current data for their review. Ultimately, the DSMB will make their own decision, irrespective of any stopping guidelines.

12.7.2. Interim Monitoring and Safety Review

A comprehensive Study Monitoring and Statistical Analysis Plan describes the types, content, and distribution schedules of study reports, and to define the statistical analysis plan to be implemented at the end of the study for the primary and secondary endpoints, including the specification of pre-defined subgroups. The purpose of the monitoring portion of the plan is to:

- Protect and ensure the safety of the subjects;
- Ensure the validity and integrity of the data for the clinical trial;
- Ensure that the clinical trial is monitored appropriately;
- Ensure that the data collected will be usable to monitor safety and address the protocol objectives; and
- Ensure that the trial steering committee and sponsor are aware of the schedule of monitoring for the clinical trial

The **Monitoring Plan** includes:

1. Progress Reports, with submission to NIAID DAIDS medical officer at approximately monthly intervals.
2. Quarterly Clinical Quality Management Plan (CQMP) Report
3. Unexpected Serious Adverse Event Reports
4. Open DSMB Report
5. Closed DSMB Report

The **Progress Reports** will include:

Table 1: Participant Accrual
Table 2: Follow-up Status for Enrolled Participants
Table 3: Participant Baseline Characteristics
Table 4: Study Drug Administration Timing and Proper Randomization
Table 5: Summary of Grade 3, 4, and Grade 5 Adverse Events and Serious AEs
Table 6: Line Listing of Cumulative Clinical Adverse Events by Participant ID
Table 7: Summary of Laboratory Abnormalities by Visit

The **DSMB Reports** will additionally include:

Table 8: Summary of EKG QTc Findings

12.7.3. Quality Control and Quality Assurance

Ongoing data quality control (QC) and quality assurance (QA) activities will occur via data management system. **Clinical Quality Management Plan** (CQMP) reports will include:

Table 9: Cumulative Withdrawn Consent and Protocol Violations
Table 9a: Line Listing of Protocol Violations by Participant ID
Table 10: Summary of Data Completeness

Table 11: Case Report Form (CRF) Delinquency Report

Table 11a: Delinquent CRFs by Participant ID

12.7.4. Trial Safety Committee

A Trial Safety Committee will be assembled to review:

- Unexpected Adverse Events
- Potential Sertraline-related Adverse Events
- Early Mortality + SAE Independent Review (Section 12.7.5).

The composition of the Trial Safety Committee shall include three physicians with experience in clinical trials and HIV medicine:

- Noeline Nakasujja – Chair, Department of Psychiatry, Makerere University
- Jason Baker – Hennepin County Medical Center, MN
- Tihana Bicanic, University of London - St. George's, UK

A quorum of 3 members is required for the early safety independent review. For adjudication of unexpected adverse events or potential sertraline-related AEs, adjudication by at least two members is necessary with consensus agreement. **The safety committee may request the unblinding of unexpected AE events or possible sertraline-related AEs from the trial biostatistician**, which would be particularly relevant if the event is potentially related to sertraline. Any individual participant unblinding would occur after the committee's adjudication, so as to not influence their adjudication.

The Trial Safety Committee shall be empowered to: 1) pause or halt further enrollment; 2) request an early DSMB review; and/or 3) request further interim safety reviews. The Trial Safety Committee shall be empowered until the DSMB assumes the responsibility for ongoing trial monitoring. The Trial Safety Committee may also choose to make recommendations to the DSMB for safety monitoring, based on their expertise in cryptococcosis, psychiatry, and clinical trials in Africa. If a replacement Trial Safety Committee member is needed, the NIH sponsor will be notified.

12.7.5. Early Safety Independent Review (Phase II activity)

To address possible concerns that there may be unanticipated safety concerns with adjunctive sertraline in addition to the standard of care, an external Trial Safety Committee will conduct enhanced safety reviews as part of an integrated Phase II trial design. There will be two a priori triggers of:

- $\geq$5% sertraline-related probable/definite adverse event (Grade 3-5 or SAE)

  This trigger would occur at 1 or more participants in the first 40 randomized (n=20 on sertraline), $\geq$2 participants in the first 80, or $\geq$3 participants in the first 120. Sertraline related Grade 3-5 adverse events are not expected. Potentially sertraline-related adverse events will be adjudicated on an ongoing basis.

- Cumulative incidence of deaths + SAEs combined over both treatment

groups of >50%. The expected incidence is 40% with the current standard of care. This composite safety outcome will be calculated by the trial biostatistician after every 40 subjects have been enrolled and accrued 3 months of follow up data. A pooled summary report shall be provided to the Trial Safety Committee for their review.

If either trigger is reached, options for the Trial Safety Committee would be a recommendation for pausing further enrollment, requesting an early DSMB review (by randomization group), and/or interim re-assessment prior to the DSMB (based on the timetable of the DSMB).

These safety reviews will continue every 40 subjects until the DSMB assumes responsibility for safety monitoring. After ~100 subjects have been enrolled, sufficient subject outcomes should allow the DSMB to judge the frequency of further reviews by the DSMB and/or Trial Safety Committee.

At the end of the phase II activities, the DSMB will be asked to make a recommendation regarding safety. Specifically, 1) should the phase III trial continue onwards; 2) is the safety monitoring adequate? and 3) whether the frequency of safety monitoring should be increased, remain the same, or decreased based on the accumulating data.

This trigger is meant for subject safety; however, there is a chance of a false positive trigger because of early anomalous results caused by random probability and/or the unequal distribution of the first few subjects. At the first review after 80 subjects, there is a 6.3% chance of a false-positive review being triggered if the true mortality + SAE incidence is 40% as anticipated, and a 59% chance of review if the underlying true mortality + SAE incidence is 50%. At reviews after 100 subjects, there is a 2.7% chance of a review being anomalously triggered if the true mortality + SAE incidence is 40%.

13. Study Monitoring, Auditing, and Inspecting

13.1. Study Monitoring Plan

Site monitors under contract to the National Institute of Allergy and Infectious Diseases (NIAID) and NIAID staff (or designee) will visit participating clinical research sites to review participants records, including consent forms, CRFs, medical records (e.g., physicians' progress notes, nurses' notes, individuals' hospital charts), and laboratory records to ensure protection of study participants, compliance with the IRB-approved protocol / amendments, and accuracy and completeness of records. Remote monitoring via internet-based review of CRFs is also a possible monitoring strategy. The monitors will inspect sites' regulatory files to ensure that local regulatory requirements, in addition to U.S. Federal regulations, are being followed. They will also inspect sites' pharmacies to review product storage and management.

An internal study team "Clinical Quality Monitoring Plan" (CQMP) will be implemented for QC/QA of CRFs to monitor correct completion of CRFs, missing CRFs, and remote review of digitally scanned source documents. Refer to CQMP for details.

13.2. Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, NIH NIAD DAIDS and DAIDS-affiliated personnel, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices. The NIH NIAID DAIDS Monitors will be used for monitoring.

13.3. Laboratory Quality Control

All protocol specific laboratory monitoring will be performed in a DAIDS-approved laboratory adhering to GCLP standards. Inclusion/Exclusion criteria of ALT and pregnancy tests may be performed via FDA-approved assays which are CLIA-waived, as per the written SOP.

14. DATA HANDLING AND RECORDKEEPING

This trial will use the DataFax system for multisite data collection. The DataFax System is a data collection system used for all data collection and data management purposes. The DataFax unit is located at the Infectious Disease Institute in Kampala. The DataFax system relies on paper CRFs to collect data during patient visits and internet-enabled fax machines to send data into a central NIH server with recognition of the CRF data via optical character recognition. The server resides at the NIH in Bethesda. Data from the CRFs are collected, reviewed, and processed by the DataFax central office, housed at the IDI in Kampala, Uganda. The DataFax system is used to assess errors on the CRFs and perform additional quality control and quality assurance procedures. The DataFax system is also utilized by data managers to develop study-specific reports. This enables real time quality assurance for the multi-site study. Individual sites receive approximately weekly error queries for missing CRFs or labs, protocol deviations, and overdue visits. The system incorporates real time error checking such that weekly error reports enable ongoing quality assurance measures.

14.1. Data Management Responsibilities

A data manager at the University of Minnesota will work with the trial statistician and DataFax unit to conduct Quality Assurance activities and prepare reports.

14.2. Essential/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, pharmacy dispensing records, recorded data from automated instruments, digital media, radiographs, and subject files and records kept at the pharmacy, at the laboratories.

For this trial, the source documents will be the CRFs completed by study staff. It is acceptable to use CRFs as source documents, when the study personnel are making their original observations and recording on CRFs. In the event of transcription from another data source, e.g. laboratory report or hospitalization records, these records are the source document. A copy of any external record should be obtained and stored in the study chart.

Routine clinic records completed by non-study staff are **not** source documents, unless they are being used to transcribe data.

15. HUMAN SUBJECTS PROTECTIONS

This study is to be conducted according to US and international standards of Good Clinical Practice (International Conference on Harmonization guidelines), Declaration of Helsinki, and International Ethical Guidelines for Biomedical Research Involving Human Subjects, applicable government regulations for Uganda, U.S., international and Institutional research policies and procedures. All investigators must have received human subject protection certification prior to human subject involvement. Study personnel involved in collecting clinical data should have current Good Clinical Practice (GCP) training.

15.1. Institutional Review Board/Ethics Committee

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 IRB of record. Likewise, any future amendments to the study protocol will be approved by each site IRB. Please refer to Appendix A for informed consent documents.

This protocol and any amendments will undergo review and approval by the institutional human subjects review boards (IRBs) at the Joint Clinical Research Centre (FWA00009772), and the Uganda National Council of Science and Technology (UNCST) under FWA00001293, University of Minnesota (FWA00000312), and other local IRBs and ethics committees as appropriate.

15.2. Vulnerable Participants

- Illiterate participants: Eligible with 3rd person impartial witness
- Pregnant women and fetuses: Not eligible to participate.
- Prisoners: Not eligible to participate.
- Children <18 years of age: Not eligible to participate.

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.

15.3. Informed Consent

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual' study participation. Upon clinic entry, persons provide a written consent for HIV treatment, which includes passive aggregate summary data collection for clinic reporting purposes. As the intervention of CrAg screening is a WHO recommendation, this would be considered standard of care – thus this alone would not require informed consent.

The regulatory purpose of obtaining informed consent would be for participation in an experimental randomized clinical trial of the preemptive treatment of CrAg+ persons with sertraline vs. placebo.

Extensive discussion of risks and possible benefits of this therapy will be provided to the subjects and their families. Consent forms describing in detail the study interventions/products, study procedures, and risks are given to the subject and written documentation of informed consent is required prior to starting intervention/administering study product. Consent forms will be IRB-approved and the subject will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the subject and answer any questions that may arise. The subjects will sign the informed consent document prior to any procedures being done specifically for the study. The subjects should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. We will assess participant comprehension during the consent process.

The subjects may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

If subjects decline to participate in the study or withdraw consent, they will still be recommended to have preemptive therapy as per WHO treatment recommendations (i.e. the fluconazole preemptive treatment regimen).

15.3.1. Documentation of 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 CrAg+ diagnosis and eligibility criteria are confirmed by a study investigator, the study investigator will obtain full informed consent for the study by providing potential subjects with an approved consent form in English, Luganda (e.g. Kampala), Runyankole (e.g. Mbarara). Informed consent must occur within 7 calendar days of the potential study participant receiving their first dose of fluconazole. 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

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 be recommended to receive standard fluconazole preemptive therapy as per WHO and national guidelines.

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 meningitis. 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 and/or refer the patient to hospital for a diagnostic lumbar puncture.

15.3.2. Literacy / Proxy Consent

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.

15.4. Waiver of Informed Consent

Cryptococcal antigen screening is considered routine care as recommended by national and WHO guidelines. Thus CrAg screening is not considered a research activity, and specific consent will not be obtained.

Two activities are requested for a waiver of informed consent. First, a waiver of consent is requested for sample storage of the initial plasma specimens from CD4 testing among persons who are CrAg+ but who do not return to enroll in the study. These previously collected specimens would otherwise be discarded. As untreated cryptococcal antigenemia has ~100% mortality, these persons most likely will have died. The purpose of storing such specimens is to investigate potential biomarkers, which are associated with high risk of acute mortality and thereby would constitute a critical value and medical emergency. Persons who decline informed consent would not have their baseline plasma stored.

Second, a waiver of informed consent is requested for testing the initial plasma/serum specimen among CrAg+ persons for ALT, which is an exclusion criterion. The purpose to do this testing is to streamline the enrollment process and to expedite initiating therapy for this life threatening cryptococcal infection. In the absence of this ALT testing, CrAg+ persons will need to return to clinic, consent for the study, have their ALT drawn, and then return to clinic for a second visit to enroll in the study and be randomized and initiate therapy. This delay would likely increase the risk of death for CrAg+ participants.

15.5. Stored Samples and Associated Data Considerations

Long term storage of specimens beyond the duration of the project requires additional written informed consent for 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 for the purposes of this research only. This does not require additional consent, 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.

15.6. Risks

Persons who are CrAg+ with CD4<100 have an expected 20-30% 6-month mortality with preemptive treatment as per international standard of care as recommended by the Ugandan, WHO, and U.S. DHHS Guidelines. Comparatively, the risk of additional sertraline vs. placebo is minimal.

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.[102] Sertraline has minimal inhibitory effects on the major cytochrome P450 enzymes, and few drug-drug interactions of clinical significance have been documented (Section 6.4).[50] Abundant clinical data supports the safety of long-term use of this as an antidepressant at doses between 50-200mg.[47-49] Like other SSRI, sertraline is relatively safe in over-dosage.[42, 43] Efavirenz decreases sertraline levels by 25-50% at lower doses of sertraline; however, there is no sertraline effect on antiretroviral medicines.[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), drowsiness (13% vs. 7% for placebo), diarrhea (20% vs. 10% for placebo), constipation (6% vs 4% for placebo), dry mouth (14% vs. 8% for placebo), dizziness (12% vs. 7% for placebo), tremor (8% vs. 2% for placebo) and decreased libido (6% vs. 1% for placebo).[51] 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.[52] Akathisia (i.e. restlessness) caused by sertraline was observed in 16% of patients in a case series.[53] Akathisia typically begins several hours after the initiation of treatment or a dose increase and usually disappears after being

15.7. Benefits

Research Participants will receive more oversight of their cryptococcal infection than in usual care. Participants who develop symptomatic meningitis will receive prompt referral for further expert care for cryptococcal meningitis in accordance with international standards of care.[13]

Participants who receive sertraline, if effective, may receive a survival benefit. There may additionally be a therapeutic benefit of more rapid resolution of depressive symptoms among persons with depression; however, this has unknown efficacy in this population with advanced HIV/AIDS and early disseminated cryptococcal infection.

15.8. Compensation

Participants will receive reimbursement of transportation expenses for unscheduled or scheduled study visits. If participants are too ill to attend clinic an unmarked vehicle (e.g. special hire) will be sent to retrieve the participant to bring them to clinic or hospital. Clinical trial insurance exists to compensate participants if they suffer bodily injury as a consequence of sertraline therapy via Lion Assurance Company, Ltd. P.O. Box 7658, Kampala.

15.9. Participant Privacy and Confidentiality

All participant-related information including case report forms, laboratory specimens, evaluation forms, reports, etc., will be kept strictly confidential. All records will be kept in a secure, double locked location and only research staff will have access to the records. Participants will be identified only by means of a coded number specific to each participant.

HIV clinic records will kept in the local HIV clinic as per local practice.

All computerized databases will identify participants by numeric codes only, and will be password-protected. Upon request, participant records will be made available to the study sponsor, the sponsor's monitoring representative, representatives of a participating pharmaceutical sponsor and applicable regulatory entities, including the UNCST, Uganda National Drug Authority, US FDA, JCRC IRB, or University of Minnesota.

15.10. New / Incidental Findings

Relevant new findings regarding CrAg screening will be incorporated by amendment into the protocol.

15.11. Study Discontinuation

The study may be discontinued at any time by the IRB, NIAID, FDA, Uganda National Drug Authority, or other government entities as part of their duties to ensure that research participants are protected.

15.12. Post-Trial Access

Participants with severe depression may elect to switch to open-label sertraline after 16 weeks at provider discretion, and participants continued beyond the end of the study period. Sertraline is a licensed / registered medicine in Uganda and is available in retail pharmacies.

15.13. Community Advisory Board and Other Relevant Stakeholders

The local Community Advisory Board(s) (e.g. "IDI Friend's Council") will be approached for this study to inform them of the national guidelines regarding pre-ART CrAg screening and this randomized clinical trial.

16. ADMINISTRATIVE PROCEDURES

16.1. Protocol Registration

Prior to implementation of this protocol, and any subsequent full version amendments, each site must have the protocol and the protocol consent form(s) approved, as appropriate, by their local institutional review board (IRB)/ethics committee (EC) and any other applicable regulatory entity (RE). Upon receiving final approval, sites will submit all required protocol registration documents to the DAIDS Protocol Registration Office (PRO) at the Regulatory Support Center (RSC). The DAIDS PRO will review the submitted protocol registration packet to ensure that all of the required documents have been received.

Site-specific informed consent forms (ICFs) WILL be reviewed and approved by the DAIDS PRO, and sites will receive an Initial Registration Notification from the DAIDS PRO that indicates successful completion of the protocol registration process. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

Upon receiving final IRB/EC and any other applicable RE approvals for an amendment sites, should implement the amendment immediately. Sites are required to submit an amendment registration packet to the DAIDS PRO at the RSC. The DAIDS PRO will review the submitted protocol registration packet to ensure that all the required documents have been received. Site-specific ICF(s) WILL NOT be reviewed and approved by the DAIDS PRO, and sites will receive an Amendment Registration Notification when the DAIDS PRO receives a complete registration packet. A copy of the Amendment Registration Notification should be retained in the site's regulatory files.

For additional information on the protocol registration process and specific documents required for initial and amendment registrations, refer to the current version of the DAIDS Protocol Registration Manual.

For additional information, refer to the protocol registration documents located at www.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/Documents/protocolregpolicy.pdf. For questions regarding protocol registration, contact the Protocol Registration Office via e-mail at protocol@tech-res.com, fax (800-418-3544 or 301-897-1701), or phone (301-897-1707).

16.2. Regulatory Oversight

The local IRB of record is the JCRC IRB in Kampala, Uganda for oversight and reporting of unexpected SAEs. Expected SAEs (e.g. meningitis, death) will be reported with annual reporting.

16.3. ClinicalTrials.gov

This protocol is subject to the Food and Drug Administration (FDA) Amendments Act of 2007 (FDAAA) and is registered in ClinicalTrials.gov with annual updates to be provided. FDA Investigational New Drug (IND) application number is: 133772.

16.4. Conflict of Interest

No conflict of interest exists by any investigator. Any investigator who develops a new conflict of interest will disclose this to their relevant University oversight board, to the executive committee, and to the Study Sponsor. Sertraline is a generic medicine, so this is not expected.

17. REFERENCES

1. Liechty CA, Solberg P, Were W, Ekwaru JP, Ransom RL, Weidle PJ, Downing R, Coutinho A, Mermin J. Asymptomatic serum cryptococcal antigenemia and early mortality during antiretroviral therapy in rural Uganda. *Trop Med Int Health*. 2007;12:929-35.
2. French N, Gray K, Watera C, Nakiyingi J, Lugada E, Moore M, Lalloo D, Whitworth JA, Gilks CF. Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. *AIDS*. 2002;16:1031-8.
3. Meya DB, Manabe YC, Castelnuovo B, Cook BA, Elbireer AM, Kambugu A, Kamya MR, Bohjanen PR, Boulware DR. Cost-effectiveness of serum cryptococcal antigen screening to prevent deaths among HIV-infected persons with a CD4+ cell count < or = 100 cells/microl who start HIV therapy in resource-limited settings. *Clin Infect Dis*. 2010;51:448-55. PMCID: 2946373.
4. Jarvis JN, Lawn SD, Vogt M, Bangani N, Wood R, Harrison TS. Screening for cryptococcal antigenemia in patients accessing an antiretroviral treatment program in South Africa. *Clin Infect Dis*. 2009;48:856-62. PMCID: 2875173.
5. Tassie JM, Pepper L, Fogg C, Biraro S, Mayanja B, Andia I, Paugam A, Priotto G, Legros D. Systematic screening of cryptococcal antigenemia in HIV-positive adults in Uganda. *J Acquir Immune Defic Syndr*. 2003;33:411-2.
6. Desmet P, Kayembe KD, De Vroey C. The value of cryptococcal serum antigen screening among HIV-positive/AIDS patients in kinshasa, zaire. *AIDS*. 1989;3:77-8.
7. Mamoojee Y, Shakoor S, Gorton RL, Sarfo S, Appiah LT, Norman B, Balakrishnan I, Phillips R, Chadwick D. Short communication: Low seroprevalence of cryptococcal antigenaemia in patients with advanced HIV infection enrolling in an antiretroviral programme in Ghana. *Trop Med Int Health*. 2011;16:53-6.
8. Pongsai P, Atamasirikul K, Sungkanuparph S. The role of serum cryptococcal antigen screening for the early diagnosis of cryptococcosis in HIV-infected patients with different ranges of CD4 cell counts. *J Infect*. 2010;60:474-7.
9. Parkes-Ratanshi R, Wakeham K, Levin J, Namusoke D, Whitworth J, Coutinho A, Mugisha NK, Grosskurth H, Kamali A, Lalloo DG. Primary prophylaxis of cryptococcal disease with fluconazole in HIV-positive Ugandan adults: A double-blind, randomised, placebo-controlled trial. *Lancet Infect Dis*. 2011;11:933-41.
10. Micol R, Lortholary O, Sar B, Laureillard D, Ngeth C, Dousset JP, Chanroeun H, Ferradini L, Guerin PJ, Dromer F, Fontanet A. Prevalence, determinants of positivity, and clinical utility of cryptococcal antigenemia in cambodian HIV-infected patients. *J Acquir Immune Defic Syndr*. 2007;45:555-9.
11. Jarvis JN, Meintjes G, Wood R, Harrison TS. Testing but not treating: Missed opportunities and lost lives in the South African antiretroviral therapy programme. *AIDS*. 2010;24:1233-5. PMCID: 2872163.
12. Letang E, Muller MC, Ntamatungiro AJ, Kimera N, Faini D, Furrer H, Battegay M, Tanner M, Hatz C, Boulware DR, Glass TR. Cryptococcal antigenemia in immunocompromised HIV patients in rural Tanzania: A preventable cause of early mortality. *Open Forum Infect Dis*. 2015;2:ofv046. PMCID: 4511744.
13. World Health Organization HAP. Rapid advice: Diagnosis, prevention and management of cryptococccal disease in HIV-infected adults, adolescents and children. 2011.
14. PEPFAR. The President's Emergency Plan for AIDS Relief: Fy 2013 technical

considerations. 2013 [cited 2015 April 1]; Available from: www.pepfar.gov/documents/organization/199147.pdf.
15. Jarvis JN, Harrison TS, Lawn SD, Meintjes G, Wood R, Cleary S. Cost effectiveness of cryptococcal antigen screening as a strategy to prevent HIV-associated cryptococcal meningitis in South Africa. *PLoS One*. 2013;8:e69288. PMCID: 3716603.
16. Butler EK, Boulware DR, Bohjanen PR, Meya DB. Long term 5-year survival of persons with cryptococcal meningitis or asymptomatic subclinical antigenemia in Uganda. *PLoS One*. 2012;7:e51291. PMCID: 3519582.
17. Mfinanga S, Chanda D, Kivuyo SL, Guinness L, Bottomley C, Simms V, Chijoka C, Masasi A, Kimaro G, Ngowi B, Kahwa A, Mwaba P, Harrison TS, Egwaga S, Jaffar S. Cryptococcal meningitis screening and community-based early adherence support in people with advanced HIV infection starting antiretroviral therapy in Tanzania and Zambia: An open-label, randomised controlled trial. *Lancet*. 2015;385:2173-82.
18. Feldmesser M, Harris C, Reichberg S, Khan S, Casadevall A. Serum cryptococcal antigen in patients with AIDS. *Clin Infect Dis*. 1996;23:827-30.
19. Yuen C, Graziani A, Pietroski N, Macgregor R, Schuster M. Cryptococcal antigenemia in HIV-infected patients [abstract no. 93]. *Clin Infect Dis*. 1994;19:579.
20. Longley N, Muzoora C, Taseera K, Mwesigye J, Rwebembera J, Chakera A, Wall E, Andia I, Jaffar S, Harrison TS. Dose response effect of high-dose fluconazole for HIV-associated cryptococcal meningitis in southwestern Uganda. *Clin Infect Dis*. 2008;47:1556-61.
21. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Recommendations for the public health approach, 2nd edition. Geneva: WHO; 2016 [cited 2016 22 Nov]; Available from: http://www.who.int/hiv/pub/arv/arv-2016/en/.
22. World Health Organization. Rapid advice: Diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children. Geneva: World Health Organization; 2011 [cited 2015 1 Nov]; Available from: www.who.int/hiv/pub/cryptococcal_disease2011.
23. Okongo M, Morgan D, Mayanja B, Ross A, Whitworth J. Causes of death in a rural, population-based human immunodeficiency virus type 1 (HIV-1) natural history cohort in Uganda. *Int J Epidemiol*. 1998;27:698-702.
24. Castelnuovo B, Manabe YC, Kiragga A, Kamya M, Easterbrook P, Kambugu A. Cause-specific mortality and the contribution of immune reconstitution inflammatory syndrome in the first 3 years after antiretroviral therapy initiation in an urban African cohort. *Clin Infect Dis*. 2009;49:965-72.
25. Corbett EL, Churchyard GJ, Charalambos S, Samb B, Moloi V, Clayton TC, Grant AD, Murray J, Hayes RJ, De Cock KM. Morbidity and mortality in South African gold miners: Impact of untreated disease due to human immunodeficiency virus. *Clin Infect Dis*. 2002;34:1251-8.
26. Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *AIDS*. 2009;23:525-30.
27. Merry M, Boulware DR. Cryptococcal meningitis treatment strategies affected by the explosive cost of flucytosine in the United States: A cost-effectiveness analysis. *Clin Infect Dis*. 2016;62:1564-8. PMCID: 4885648.
28. Nayak R, Xu J. Effects of sertraline hydrochloride and fluconazole combinations on Cryptococcus neoformans and Cryptococcus gattii. *Mycology*. 2010;1:99-105.

29. Zhai B, Wu C, Wang L, Sachs MS, Lin X. The antidepressant sertraline provides a promising therapeutic option for neurotropic cryptococcal infections. *Antimicrob Agents Chemother*. 2012;56:3758-66. PMCID: 3393448.
30. Lass-Florl C, Dierich MP, Fuchs D, Semenitz E, Ledochowski M. Antifungal activity against candida species of the selective serotonin-reuptake inhibitor, sertraline. *Clin Infect Dis*. 2001;33:E135-6.
31. Lass-Florl C, Dierich MP, Fuchs D, Semenitz E, Jenewein I, Ledochowski M. Antifungal properties of selective serotonin reuptake inhibitors against Aspergillus species in vitro. *J Antimicrob Chemother*. 2001;48:775-9.
32. Smith KD, Achan B, Hullsiek KH, McDonald TR, Okagaki LH, Alhadab AA, Akampurira A, Rhein JR, Meya DB, Boulware DR, Nielsen K. Increased antifungal drug resistance in clinical isolates of *Cryptococcus neoformans* in Uganda. *Antimicrob Agents Chemother*. 2015;59:7197-204. PMCID: 4649209.
33. Rhein J, Morawski BM, Hullsiek KH, Nabeta HW, Kiggundu R, Tugume L, Musubire A, Akampurira A, Smith KD, Alhadab A, Williams DA, Abassi M, Bahr NC, Velamakanni SS, Fisher J, Nielsen K, Meya DB, Boulware DR. Efficacy of adjunctive sertraline for the treatment of HIV-associated cryptococcal meningitis: An open-label dose-ranging study. *Lancet Infect Dis*. 2016;16:809-18. PMCID: 4927382.
34. Trevino-Rangel Rde J, Villanueva-Lozano H, Hernandez-Rodriguez P, Martinez-Resendez MF, Garcia-Juarez J, Rodriguez-Rocha H, Gonzalez GM. Activity of sertraline against *Cryptococcus neoformans*: In vitro and in vivo assays. *Med Mycol*. 2016;54:280-6.
35. Boulware DR, Meya DB, Muzoora C, Rolfes MA, Huppler Hullsiek K, Musubire A, Taseera K, Nabeta HW, Schutz C, Williams DA, Rajasingham R, et al. Timing of antiretroviral therapy after diagnosis of cryptococcal meningitis. *N Engl J Med*. 2014;370:2487-98. PMCID: 4127879.
36. Sloan D, Dlamini S, Paul N, Dedicoat M. Treatment of acute cryptococcal meningitis in HIV infected adults, with an emphasis on resource-limited settings. *Cochrane Database Syst Rev*. 2008:CD005647.
37. Ruiz NM, Labriola DF, Fiske WD, Joshi AS, Manion DJ, Villano SA. Efavirenz plasma levels and therapeutic response are affected in patients concomitantly receiving selective serotonin reuptake inhibitors. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy; September 17-20; Toronto, Canada2000.
38. Markowitz JS, DeVane CL. Rifampin-induced selective serotonin reuptake inhibitor withdrawal syndrome in a patient treated with sertraline. *J Clin Psychopharmacol*. 2000;20:109-10.
39. Ninan PT, Koran LM, Kiev A, Davidson JR, Rasmussen SA, Zajecka JM, Robinson DG, Crits-Christoph P, Mandel FS, Austin C. High-dose sertraline strategy for nonresponders to acute treatment for obsessive-compulsive disorder: A multicenter double-blind trial. *J Clin Psychiatry*. 2006;67:15-22.
40. Pfizer. Zoloft prescribing information for the u.S. In: Pfizer, editor. FDA Package Insert2012.
41. Saletu B, Grunberger J, Linzmayer L. On central effects of serotonin re-uptake inhibitors: Quantitative eeg and psychometric studies with sertraline and zimelidine. *J Neural Transm*. 1986;67:241-66.
42. Barbey JT, Roose SP. SSRI safety in overdose. *J Clin Psychiatry*. 1998;59 Suppl 15:42-8.
43. Isbister GK, Bowe SJ, Dawson A, Whyte IM. Relative toxicity of selective serotonin

reuptake inhibitors (ssris) in overdose. *J Toxicol Clin Toxicol*. 2004;42:277-85.
44. Nelson LS, Erdman AR, Booze LL, Cobaugh DJ, Chyka PA, Woolf AD, Scharman EJ, Wax PM, Manoguerra AS, Christianson G, Caravati EM, Troutman WG. Selective serotonin reuptake inhibitor poisoning: An evidence-based consensus guideline for out-of-hospital management. *Clin Toxicol (Phila)*. 2007;45:315-32.
45. Williams DA, Kiiza T, Kwizera R, Kiggundu R, Velamakanni S, Meya DB, Rhein J, Boulware DR. Evaluation of fingerstick cryptococcal antigen lateral flow assay in HIV-infected persons: A diagnostic accuracy study. *Clin Infect Dis*. 2015;61:464-7. PMCID: 4503809.
46. Boulware DR, Rolfes MA, Rajasingham R, von Hohenberg M, Qin Z, Taseera K, Schutz C, Kwizera R, Butler EK, Meintjes G, Muzoora C, Bischof JC, Meya DB. Multisite validation of cryptococcal antigen lateral flow assay and quantification by laser thermal contrast. *Emerg Infect Dis*. 2014;20:45-53. PMCID: 3884728.
47. Cook EH, Wagner KD, March JS, Biederman J, Landau P, Wolkow R, Messig M. Long-term sertraline treatment of children and adolescents with obsessive-compulsive disorder. *J Am Acad Child Adolesc Psychiatry*. 2001;40:1175-81.
48. Rapaport MH, Wolkow R, Rubin A, Hackett E, Pollack M, Ota KY. Sertraline treatment of panic disorder: Results of a long-term study. *Acta Psychiatr Scand*. 2001;104:289-98.
49. Rasmussen S, Hackett E, DuBoff E, Greist J, Halaris A, Koran LM, Liebowitz M, Lydiard RB, McElroy S, Mendels J, O'Connor K. A 2-year study of sertraline in the treatment of obsessive-compulsive disorder. *Int Clin Psychopharmacol*. 1997;12:309-16.
50. Naranjo CA, Sproule BA, Knoke DM. Metabolic interactions of central nervous system medications and selective serotonin reuptake inhibitors. *Int Clin Psychopharmacol*. 1999;14 Suppl 2:S35-47.
51. Pfizer. Zoloft prescribing information for the u.S" (pdf). In: Pfizer, editor.2012.
52. Fernandez-Banares F, Esteve M, Espinos JC, Rosinach M, Forne M, Salas A, Viver JM. Drug consumption and the risk of microscopic colitis. *Am J Gastroenterol*. 2007;102:324-30.
53. Olivera AO. Sertraline and akathisia: Spontaneous resolution. *Biol Psychiatry*. 1997;41:241-2.
54. Siepmann M, Grossmann J, Muck-Weymann M, Kirch W. Effects of sertraline on autonomic and cognitive functions in healthy volunteers. *Psychopharmacology (Berl)*. 2003;168:293-8.
55. Gorenstein C, de Carvalho SC, Artes R, Moreno RA, Marcourakis T. Cognitive performance in depressed patients after chronic use of antidepressants. *Psychopharmacology (Berl)*. 2006;185:84-92.
56. Stone M, Laughren T, Jones ML, Levenson M, Holland PC, Hughes A, Hammad TA, Temple R, Rochester G. Risk of suicidality in clinical trials of antidepressants in adults: Analysis of proprietary data submitted to US food and drug administration. *BMJ*. 2009;339:b2880. PMCID: 2725270.
57. Rosenbaum JF, Fava M, Hoog SL, Ascroft RC, Krebs WB. Selective serotonin reuptake inhibitor discontinuation syndrome: A randomized clinical trial. *Biol Psychiatry*. 1998;44:77-87.
58. Bandelow B, Behnke K, Lenoir S, Hendriks GJ, Alkin T, Goebel C, Clary CM. Sertraline versus paroxetine in the treatment of panic disorder: An acute, double-blind noninferiority comparison. *J Clin Psychiatry*. 2004;65:405-13.

59. Lewis RJ, Angier MK, Williamson KS, Johnson RD. Analysis of sertraline in postmortem fluids and tissues in 11 aviation accident victims. *J Anal Toxicol*. 2013;37:208-16.
60. Psaros C, Haberer JE, Katabira E, Ronald A, Tumwesigye E, Campbell JD, Wangisi J, Mugwanya K, Kintu A, Enyakoit M, Thomas KK, Donnell D, Krows M, Kidoguchi L, Ware N, Baeten JM, Celum C, Bangsberg DR, Safren SA. An intervention to support HIV preexposure prophylaxis adherence in HIV-serodiscordant couples in Uganda. *J Acquir Immune Defic Syndr*. 2014;66:522-9. PMCID: 4191829.
61. Wiens MO, MacLeod S, Musiime V, Ssenyonga M, Kizza R, Bakeera-Kitaka S, Odoi-Adome R, Ssali F. Adherence to antiretroviral therapy in HIV-positive adolescents in Uganda assessed by multiple methods: A prospective cohort study. *Paediatr Drugs*. 2012;14:331-5.
62. Foisy MM, Yakiwchuk EM, Hughes CA. Induction effects of ritonavir: Implications for drug interactions. *Ann Pharmacother*. 2008;42:1048-59.
63. Masur H, Brooks JT, Benson CA, Holmes KK, Pau AK, Kaplan JE. Prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: Updated guidelines from the centers for disease control and prevention, National Institutes of Health, and HIV medicine association of the Infectious Diseases Society of America. *Clin Infect Dis*. 2014;58:1308-11. PMCID: 3982842.
64. FDA. Guidance for industry: E14 clinical evaluation of qt/qtc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs 2005 [cited 2016 7 July]; Available from: www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm073153.pdf.
65. Wakeham K, Parkes-Ratanshi R, Watson V, Ggayi AB, Khoo S, Lalloo DG. Co-administration of fluconazole increases nevirapine concentrations in HIV-infected Ugandans. *J Antimicrob Chemother*. 2010;65:316-9. PMCID: 2809247.
66. Manosuthi W, Athichathanabadi C, Uttayamakul S, Phoorisri T, Sungkanuparph S. Plasma nevirapine levels, adverse events and efficacy of antiretroviral therapy among HIV-infected patients concurrently receiving nevirapine-based antiretroviral therapy and fluconazole. *BMC Infect Dis*. 2007;7:14. PMCID: PMC1828732.
67. Habtewold A, Amogne W, Makonnen E, Yimer G, Nylen H, Riedel KD, Aderaye G, Bertilsson L, Burhenne J, Diczfalusy U, Aklillu E. Pharmacogenetic and pharmacokinetic aspects of cyp3a induction by efavirenz in HIV patients. *Pharmacogenomics J*. 2013;13:484-9.
68. Mouly S, Lown KS, Kornhauser D, Joseph JL, Fiske WD, Benedek IH, Watkins PB. Hepatic but not intestinal cyp3a4 displays dose-dependent induction by efavirenz in humans. *Clin Pharmacol Ther*. 2002;72:1-9.
69. Xu C, Desta Z. In vitro analysis and quantitative prediction of efavirenz inhibition of eight cytochrome p450 (cyp) enzymes: Major effects on cyps 2b6, 2c8, 2c9 and 2c19. *Drug Metab Pharmacokinet*. 2013;28:362-71. PMCID: PMC4075192.
70. Michaud V, Ogburn E, Thong N, Aregbe AO, Quigg TC, Flockhart DA, Desta Z. Induction of cyp2c19 and cyp3a activity following repeated administration of efavirenz in healthy volunteers. *Clin Pharmacol Ther*. 2012;91:475-82. PMCID: PMC3667659.
71. von Moltke LL, Greenblatt DJ, Granda BW, Giancarlo GM, Duan SX, Daily JP, Harmatz JS, Shader RI. Inhibition of human cytochrome p450 isoforms by nonnucleoside reverse transcriptase inhibitors. *J Clin Pharmacol*. 2001;41:85-91.
72. Esteban J, Pellin Mde L, Gimeno C, Barril J, Gimenez J, Mora E, Garcia-Perez AG.

Increase of r-/s-methadone enantiomer concentration ratio in serum of patients treated with either nevirapine or efavirenz. *Drug Metab Lett*. 2008;2:269-79.
73. Drug development and drug interactions: Table of substrates, inhibitors and inducers. FDA; 2014 [cited]; Available from: http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm.
74. Erickson DA, Mather G, Trager WF, Levy RH, Keirns JJ. Characterization of the in vitro biotransformation of the HIV-1 reverse transcriptase inhibitor nevirapine by human hepatic cytochromes p-450. *Drug Metab Dispos*. 1999;27:1488-95.
75. Cottrell ML, Hadzic T, Kashuba AD. Clinical pharmacokinetic, pharmacodynamic and drug-interaction profile of the integrase inhibitor dolutegravir. *Clin Pharmacokinet*. 2013;52:981-94. PMCID: PMC3805712.
76. Kumar GN, Dykstra J, Roberts EM, Jayanti VK, Hickman D, Uchic J, Yao Y, Surber B, Thomas S, Granneman GR. Potent inhibition of the cytochrome p-450 3a-mediated human liver microsomal metabolism of a novel HIV protease inhibitor by ritonavir: A positive drug-drug interaction. *Drug Metab Dispos*. 1999;27:902-8.
77. Laboratories A. Kaletra. FDA; 2007.
78. Yeh RF, Gaver VE, Patterson KB, Rezk NL, Baxter-Meheux F, Blake MJ, Eron JJ, Jr., Klein CE, Rublein JC, Kashuba AD. Lopinavir/ritonavir induces the hepatic activity of cytochrome p450 enzymes cyp2c9, cyp2c19, and cyp1a2 but inhibits the hepatic and intestinal activity of cyp3a as measured by a phenotyping drug cocktail in healthy volunteers. *J Acquir Immune Defic Syndr*. 2006;42:52-60.
79. Wyen C, Fuhr U, Frank D, Aarnoutse RE, Klaassen T, Lazar A, Seeringer A, Doroshyenko O, Kirchheiner JC, Abdulrazik F, Schmeisser N, Lehmann C, Hein W, Schomig E, Burger DM, Fatkenheuer G, Jetter A. Effect of an antiretroviral regimen containing ritonavir boosted lopinavir on intestinal and hepatic cyp3a, cyp2d6 and p-glycoprotein in HIV-infected patients. *Clin Pharmacol Ther*. 2008;84:75-82.
80. Rathbun RC, Liedtke MD. Antiretroviral drug interactions: Overview of interactions involving new and investigational agents and the role of therapeutic drug monitoring for management. *Pharmaceutics*. 2011;3:745-81. PMCID: PMC3857057.
81. Squib BM. Atazanavir. FDA; 2003.
82. Orrick JJ, Steinhart CR. Atazanavir. *Ann Pharmacother*. 2004;38:1664-74.
83. Marzolini C, Gibbons S, Khoo S, Back D. Cobicistat versus ritonavir boosting and differences in the drug-drug interaction profiles with co-medications. *J Antimicrob Chemother*. 2016;71:1755-8.
84. Sherman EM, Worley MV, Unger NR, Gauthier TP, Schafer JJ. Cobicistat: Review of a pharmacokinetic enhancer for HIV infection. *Clin Ther*. 2015;37:1876-93.
85. Wang P, Shehu AI, Liu K, Lu J, Ma X. Biotransformation of cobicistat: Metabolic pathways and enzymes. *Drug Metab Lett*. 2016;10:111-23.
86. Moody DE, Liu F, Fang WB. Azole antifungal inhibition of buprenorphine, methadone and oxycodone in vitro metabolism. *J Anal Toxicol*. 2015;39:374-86.
87. Lehtinen T, Tolonen A, Turpeinen M, Uusitalo J, Vuorinen J, Lammintausta R, Pelkonen O, Scheinin M. Effects of cytochrome p450 inhibitors and inducers on the metabolism and pharmacokinetics of ospemifene. *Biopharm Drug Dispos*. 2013;34:387-95.
88. Sekar V, De Paepe E, De Marez T. Pharmacokinetic interaction between darunavir (tmc 114), a new protease inhibitor, and the selective serotonin reuptake inhibitors (ssris), paroxetine and sertraline (abstract p295). . 8th International Congress on Drug

Therapy in HIV Infection; Glasgow, Scotland2006.
89. Kalyesubula R, Kagimu M, Opio KC, Kiguba R, Semitala CF, Schlech WF, Katabira ET. Hepatotoxicity from first line antiretroviral therapy: An experience from a resource limited setting. *Afr Health Sci*. 2011;11:16-23. PMCID: PMC3092323.
90. Lee BE, Feinberg M, Abraham JJ, Murthy AR. Congenital malformations in an infant born to a woman treated with fluconazole. *Pediatr Infect Dis J*. 1992;11:1062-4.
91. Pursley TJ, Blomquist IK, Abraham J, Andersen HF, Bartley JA. Fluconazole-induced congenital anomalies in three infants. *Clin Infect Dis*. 1996;22:336-40.
92. Aleck KA, Bartley DL. Multiple malformation syndrome following fluconazole use in pregnancy: Report of an additional patient. *Am J Med Genet*. 1997;72:253-6.
93. Lopez-Rangel E, Van Allen MI. Prenatal exposure to fluconazole: An identifiable dysmorphic phenotype. *Birth Defects Res A Clin Mol Teratol*. 2005;73:919-23.
94. Paranyuk Y, Levine G, Figueroa R. Candida septicemia in a pregnant woman with hyperemesis receiving parenteral nutrition. *Obstet Gynecol*. 2006;107:535-7.
95. Paz A, Gonen R, Potasman I. Candida sepsis following transcervical chorionic villi sampling. *Infect Dis Obstet Gynecol*. 2001;9:147-8. PMCID: 1784650.
96. Wiesinger EC, Mayerhofer S, Wenisch C, Breyer S, Graninger W. Fluconazole in candida albicans sepsis during pregnancy: Case report and review of the literature. *Infection*. 1996;24:263-6.
97. Krcmery S, Masar O, Krcmery V, Jr. Nosocomial torulopsis glabrata fungemia with shock in a pregnant woman successfully treated with high doses of fluconazole and delivery of healthy child. *J Chemother*. 1995;7:253.
98. Haddow LJ, Colebunders R, Meintjes G, Lawn SD, Elliott JH, Manabe YC, Bohjanen PR, Sungkanuparph S, Easterbrook PJ, French MA, Boulware DR. Cryptococcal immune reconstitution inflammatory syndrome in HIV-1-infected individuals: Proposed clinical case definitions. *Lancet Infect Dis*. 2010;10:791-802. PMCID: 3026057.
99. Boulware DR, Hullsiek KH, Puronen CE, Rupert A, Baker JV, French MA, Bohjanen PR, Novak RM, Neaton JD, Sereti I. Higher levels of CRP, d-dimer, il-6, and hyaluronic acid before initiation of antiretroviral therapy (ART) are associated with increased risk of AIDS or death. *J Infect Dis*. 2011;203:1637-46. PMCID: 3096784.
100. Boulware DR, Meya DB, Bergemann TL, Wiesner DL, Rhein J, Musubire A, Lee SJ, Kambugu A, Janoff EN, Bohjanen PR. Clinical features and serum biomarkers in HIV immune reconstitution inflammatory syndrome after cryptococcal meningitis: A prospective cohort study. *PLoS Med*. 2010;7:e1000384. PMCID: 3014618.
101. Lakatos E. Sample sizes based on the log-rank statistic in complex clinical trials. *Biometrics*. 1988;44:229-41.
102. Aitken M, Berndt ER, Cutler DM. Prescription drug spending trends in the United States: Looking beyond the turning point. *Health Aff (Millwood)*. 2009;28:w151-60.

18. APPENDICES

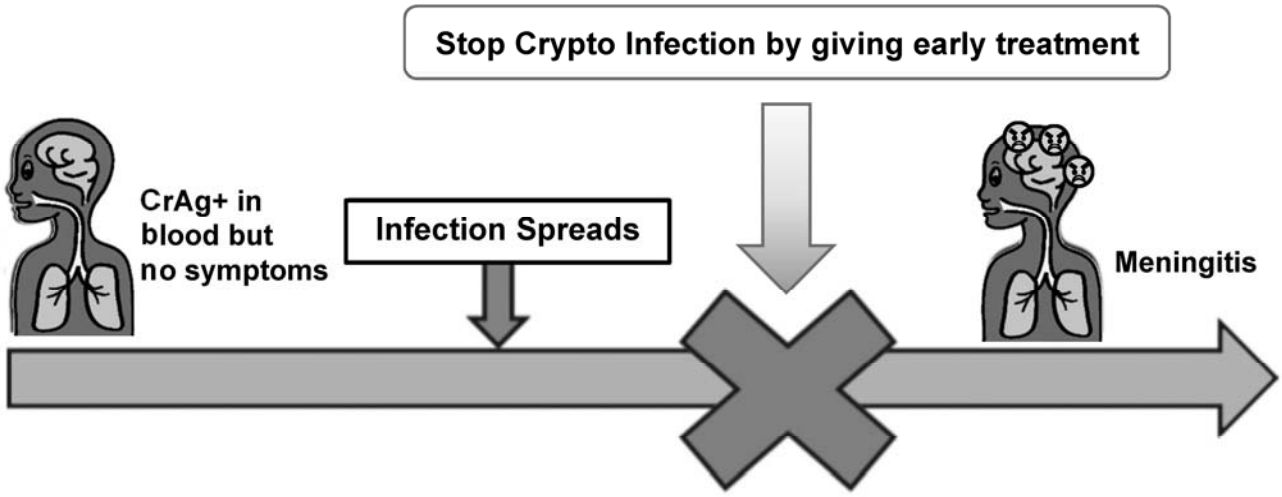
APPENDIX A: Informed Consent

Study Title: Cryptococcal Antigen Screening plus Sertraline

The lead investigators of this research study are Dr. David Meya of the Infectious Disease Institute, and Dr. Radha Rajasingham and Dr. David Boulware of the University of Minnesota USA.

Study Purpose

Cryptococcal meningitis or "Crypto" is a life threatening fungal infection around the brain that requires hospitalization for treatment for 14 days and then continued therapy. However, this infection can be detected before one develops symptoms and becomes ill. People can be screened for infection by a blood test to detect "cryptococcal antigen," (called CrAg), which is part of the fungus, in your blood. We have tested your blood and found this cryptococcal antigen (CrAg). This means that you recommended to receive early treatment now.



However, It is unknown how best to treat people with cryptococcal antigen in their blood, who don't otherwise yet have symptoms of infection around their brain. It is unknown how best to treat people with a positive blood test for "Crypto." If no treatment is given, most people will develop infection of the brain and/or die. Ugandan guidelines suggest using both HIV medicines and an anti-fungal medicine, called fluconazole, to treat this early infection. However, despite this treatment 1 in 4 people may get sick and/or die.

Researchers have recently begun to look at another medicine that may be helpful in treating crypto. This medicine is called Sertraline, and it is actually a medicine that has been used for more than 25 years to treat depression (sadness). Sertraline is one of the most commonly used medicines worldwide and is a registered, approved medicine in Uganda. Because sertraline is so widely used, much is known about this drug. It appears to be a very safe medicine that has few interactions with other medicines, and it is given as a pill. However, the ability of sertraline to treat Crypto is still being tested. The sertraline dose needed for Crypto is higher than commonly used to treat depression (sadness) at a dose of 400mg per day. However in a recent study of infection of the brain with Crypto, adding sertraline 400mg for two-weeks did not work better than standard

therapy alone. Sertraline has not been used in people without symptoms to treat early Crypto infection. We do not know if Sertraline will work in early Crypto infection.

The purpose of this study is to determine if usual therapy plus a high dose of Sertraline (400mg per day), will be better than usual therapy alone for treating early Crypto infection. Usual therapy is antifungal (fluconazole) and HIV medicines. We hope to have 600 people in Uganda participate. We will start by asking a number of questions to see if you are eligible for this research study as well as check your heart rate and rhythm with an electrocardiogram (EKG) for a baseline measurement.

You confirm that the following has been explained to you, and you have had a chance to ask questions:

1. The benefit of Sertraline (the experimental medicine) to treat Crypto infection in people is unknown. There may or may not be any benefit.
2. Sertraline is usually given to treat depression (sadness) at doses of 50-200mg per day. This study will use a dose of 400mg per day.
3. You will receive the usual antifungal medicine (fluconazole).
4. The purpose of this study is to find out if Sertraline in addition to usual medicine will be better treatment to improve survival. You will therefore be selected to be treated with:
    A. placebo (empty pill), or
    B. Sertraline (experimental medicine)
5. Whether you receive a or b (above) will be selected by chance (such as flipping a coin).
6. You will be required to take all of your medications faithfully.
7. You will be required to return for clinic visits approximately every 2 weeks x 3 visits, then every 4 weeks for 6 months.
8. You may be contacted by phone or text message as a reminder of clinic appointments, to check how you are doing, and see if you are having any problems with your medicines.
9. You will undergo further blood tests for this study today, in 1 month, in 2 months, and in 3 months. These tests will check your liver and kidney function.
10. If you agree, some samples may be collected and stored for future research tests to better understand Crypto infection and treatment. This may benefit future people in Uganda and around the world, but you will not benefit directly from this.
11. You can choose not to have your samples stored and still participate in this study.
12. Study doctors will review your medical records.
13. You will not be paid for participating in this study, but you will be given up to 20,000 Uganda shillings to refund your transport expenses for each study related visit.
14. Any information about you shall be kept private, and your data will be used without your name, picture, or identity.
15. Agreeing to participate in this study is voluntary and you can withdraw from the study at any time if you so wish by telling the study doctors. If you withdraw from the study, you can continue to receive your HIV medicines free at the clinic. You will continue to be treated with HIV medicines and fluconazole, as this is standard of care.
16. You will not be giving up any of your legal rights by signing this consent form.
17. You are going to be given a copy of this form.

18. If you develop a bad headache or become sick during the study, you should return to clinic right away or contact the study doctor by phone. If you are too sick to travel to clinic, we will help you get to hospital and provide the needed medical care.

Risks

It is recommended by the Ugandan national guidelines and your doctors that you start antifungal treatment along with antiretroviral medicines to treat the Crypto infection and your HIV infection. The study has the following risks:

There is a risk that you could be receiving an extra medicine that is not needed.

Sertraline is a relatively safe drug, but side effects can still occur, as with any medicine. Common mild side effects can include:

- Nausea (14%)
- Diarrhea (10%)
- Increased sweating (5%)
- Dizziness (5%)
- Tremor/Shakes (6%)
- Dry mouth (6%)
- Sleepiness (6%)
- Trouble falling asleep (10%)
- Restlessness
- Other side effects occur in 1 in 100 people

Most of these side effects are fairly mild and go away over 1-2 weeks on the medicine or with stopping the medicine. Severe adverse reactions occur very rarely (less than 1 in 1000 people). These rare events include sudden eye pain and bleeding. Your doctors will check blood tests for your liver and kidney function to make sure no severe problems happen. Your doctors will also check your heart rate and rhythm. Side effects may or may not be more common because of the higher sertraline dose. Until the study is completed, we will not know if sertraline is more helpful, or if more side effects occur. Stopping the Sertraline suddenly can cause side effects. Thus, if planning to stop the Sertraline, you must tell your doctor. Your doctor will tell you how to slowly decrease the amount of Sertraline by taking a little bit less each day.

Some similar drugs can cause increased thoughts of harming oneself (suicide) in adults with sadness who are <25 years old, but that this is not common with Sertraline. This can happen in about 1 in 500 people with sadness. However, we will ask you about any harmful thoughts you may have and refer you to a counsellor if this is a problem for you.

This study requires that a small amount of blood be taken through a needle from a vein in your arm several times during the study. This blood will be taken to check your liver and kidney function. If there are problems found, your medicine may be decreased. This is about 60mL (12 teaspoons) more blood in total than would have been taken over the next 6 months than if you do not participate.

This small amount of extra blood taken should have no effect on you, since your body will have time to make new blood. It is possible that there may be a small amount of bleeding or bruising around the needle site. Infection at the needle site is also a very small possibility.

Benefits of participating in the study

You may not benefit directly from participating in this research study. However, participation will provide important information about whether Sertraline is an effective treatment for cryptococcal meningitis. This may benefit other patients in the future.

Pregnancy

Pregnant and breastfeeding women **cannot** be in this research study. **Pregnancy must be avoided** when taking the usual medicine (fluconazole) for Crypto infection, due to risks to the unborn child (fetus). This risk is not unique to the study. If you are a woman and sexually active, you will be required to use at least **one** method(s) to prevent getting pregnant, such as condoms, oral contraceptive pills, etc. If you later desire to become pregnant or think you may be pregnant, please notify the study doctor right away, so that your medicines can be adjusted.

Alternatives to Study Participation

If you do not participate in this research study, you would be recommended to receive the usual fluconazole antifungal medicine and standard HIV medicines.

Sample Storage

If you agree to participate in this study, we will collect some samples of your blood for your medical care and leftover blood for research during the study. These stored samples will be identified by a number, not your name or other personal information, so that others will not be able to identify you. Testing will be for research related to Crypto infection, HIV, the immune system, and to measure medicine levels in your blood. Some of the tests will be done locally in Uganda, and some may be done in the United States. With your permission, we would like to collect a small volume of blood (up to 20mL) along with your other routine blood draws for research testing (50mL in total). If you prefer to not have any extra blood drawn, you may still participate in the study. Many research tests will be performed later and will not change your treatment, thus you will not receive the test results. The results may help future persons by better knowing about Crypto infection. This may include de-identified genetic testing, which will not be identified back to you individually.

Sample ownership and duration of storage

If you are willing to donate samples for future research, it means you are giving them to us. The samples will be under the responsibility of the researchers and may be shared with other researchers to assist with testing. You will not personally get direct benefit from this future testing, but your contribution may help others in the future. They will not be sold. There is no limit on how long your samples will be stored. However, you can change your mind at any time without penalty and tell us to destroy the samples. If you prefer that we do not keep your samples after the study is completed, you may still participate in the study.

Research Related Injury

In the event that you become sick during the study, let us know right away. We will help you get the care you need. It is very important to let us know right away if you develop a severe headache which does not go away. In this case, you may need a different treatment.

If you experience problems related to Crypto infection of the brain or HIV-infectionyou will not have to pay for it. If you experience other health problems, a referral to specialized care will be made, but this study cannot pay for health care, which is unrelated to the Crypto infection. There is no

program for compensation either through the local clinic, local University, or the U.S. National Institutes of Health (NIH). The NIH is a sponsor of this study.

Confidentiality

Your study information will be kept private, but will be included in your medical records. Your name, picture of you, or identity will not be shared in any publications or presentations. Your records for the study may be reviewed by representatives of the U.S. National Institutes of Health, the University of Minnesota, the US Federal Drug Administration, and Ugandan authorities with the appropriate regulatory oversight to assure the accuracy and quality of the records and the correct conduct of the research study.

Voluntary Nature of the Study

Participation in this study is voluntary and standard care is available. Your choice to or not to be in this study will not affect your current or future relations with the clinic. If you choose to participate, you are free to stop at any time without affecting those relationships. If you want to stop being in the study, you will continue to receive HIV medicines from the clinic, but without the additional research medicine and safety blood tests provided by the research study. If you stop being in the study, we ask to be able to call you in 6 months by telephone to see how you are doing.

Study approval

Approval to conduct this study will be granted by the Uganda National Council for Science and Technology as well as Uganda National Drug Authority. The National Institutes of Health (NIH) is the United States study funder, and the University of Minnesota is the sponsor.

Contacts and Questions

You may ask any questions you have now. Please ask. If you have questions later or any urgent health concerns, you are encouraged to contact:

- Dr. David Meya in Kampala on phone number 077-254-3730
- Dr. Conrad Muzoora in Mbarara on phone number 077-254-7175
- Dr. Fred Kibengo in Masaka on phone number 077-243-5251

You will be given a clinic appointment card with the phone number before you leave. In case of any questions regarding Welfare and rights of participants, you should contact:

> Dr. Jesse Kagimba, the Chairperson Joint Clinic Research Centre Ethics Review Committee on telephone number 0414201148/7 or the Executive Secretary Uganda National Council for Science and Technology, Plot 6 Kimera Road, Ntinda on telephone 041-4750-500.

Informed consent

I hereby consent to participate in this research: “Cryptococcal Antigen Screening plus Sertraline”

Participant Name: _____ Date _____

Participant Signature:

_____ _____
OR Thumbprint

Signature of Witness …….Date……
(If consent read aloud to the participant)

Name of Witness…….

Description of who the Witness is: ……

Name / Signature of the Person who administered this informed consent:
_____ Date/Time: _____

For More Information

A description of this clinical trial will be available on www.ClinicalTrials.gov, as required by United States Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

SAMPLE STORAGE INFORMED CONSENT FORM

STUDY TITLE: **Cryptococcal Antigen Screening plus Sertraline**

Purpose of Sample Storage

The purpose of this research study is to find out if a high dose of Sertraline in addition to standard therapy, will be tolerated, safe, and more effective than standard therapy alone for treating early Crypto infection to increase survival. We request your permission to store samples for future research, so that we may better understand early Crypto infection for others in the future. This storage of your samples will not benefit you directly, but this may benefit other Ugandans in the future.

You understand the following:

1. If you agree to participate in this study, we will collect some samples of your blood for your medical care and leftover blood for research during the study.
2. We would like to store these samples. These samples will be identified by a number, not your name or other personal information, so that only people working on the study can identify you.
3. As part of the study, we would like to keep any left-over stored samples for research testing about Crypto infection, HIV, the immune system, and to measure medicine levels in your blood.
4. In addition, we would like to keep the left-over stored samples for future possible research.
5. With your permission, we would like to collect a small volume of blood (up to 20mL) along with your other routine blood draws for research testing (50mL in total). If you prefer to not have any extra blood drawn, you may still participate in the study.
6. Many research tests will be performed later and will not change your treatment, thus you will not receive the test results. The results may help future persons by better knowing about Crypto infection. This may include anonymous genetic testing, which will not be identified back to you or your name.

Duration of storage

There is no limit on how long your samples will be stored. However, you can change your mind at any time without penalty and tell us to destroy the samples. Samples may be stored in Uganda or in the United States.

Ownership

If you are willing to donate samples for future research, it means you are giving them to us. The samples will be under the responsibility of the researchers and may be shared with other researchers to assist with testing. You will not personally receive direct benefit from this future testing, but your contribution may help others in the future. They will not be sold.

Study approval

Approval to conduct this study in Uganda has been granted by Uganda National Council for Science and Technology.

Welfare of participants

You will not be paid to have your samples stored for future biological tests.

Confidentiality

Your privacy will be protected as your samples will not have your name and any genetic information will not be put in your medical record. The identity and results from your samples will be kept private. Your identity will not be disclosed in any publications or presentations.

Voluntary Nature of the Study

You may refuse to donate your left-over samples for future research, or refuse to have additional samples collected for such future research. Both of these things are optional. Your decision will not affect your participation in this research study or your medical care in any way. If you do not wish to donate your left-over samples for future research, your samples will be destroyed when this study is finished.

Risks

Once the samples are collected, there are no additional risks to you from testing and storage for purposes of this study or future research.

Contacts and Questions

You may ask any questions you have now. If you have questions later, you are encouraged to contact:

- Dr. David Meya in **Kampala** on phone number **077-254-3730,** or
- Dr. Conrad Muzoora in **Mbarara** on phone number **077-254-7175**.
- Dr. Fred Kibengo in **Masaka** on phone number 077-243-5251

In case of any questions regarding the Welfare and rights of participants, you should contact Dr. Jesse Kagimba, the Chairperson Joint Clinical Research Centre Ethics Review Committee on telephone number 0414201148/7 or the HIV/AIDS Research Committee Secretariat at Uganda National Council for Science and Technology, Plot 6, Kimera Road, Ntinda PO Box 6884; on telephone 041-4750-500.

If you have any questions about the use of your samples, you can ask them now or contact the above individuals later.

Please indicate your choice regarding storage of your samples below.

☐ **I consent to the collection, testing and storage of my samples (blood, spinal fluid, and other samples) for the purposes of this research, as well as for future research. Samples may be stored indefinitely, unless I change my mind and ask for them to be destroyed. This may include genetic testing.**

☐ **I consent to the collection, testing and storage of my samples (blood and spinal fluid) for the purposes of this research only. Samples will be destroyed when the study is finished.**

Participant / Representative /

Parent / Guardian Signature _____**OR** ☐

Date _____ Participant Name _____

Signature of Witness (if needed): _____

(If consent read aloud to the participant)

Name of Witness……

Description of who the Witness is: …….

Name/Signature of who administered informed consent: _____

Date/Time: _____

Appendix B: Study Statistical Plan

Appendix B: Study Monitoring and Statistical Analysis Plan

Table of Contents

Purpose

The purpose of this Study Monitoring and Statistical Analysis Plan (SMSAP) is to describe the types, content, and distribution schedules of study progress and safety monitoring reports required for NIH NIAID sponsored studies, and to define the statistical analysis plan that will be implemented at the end of the study.

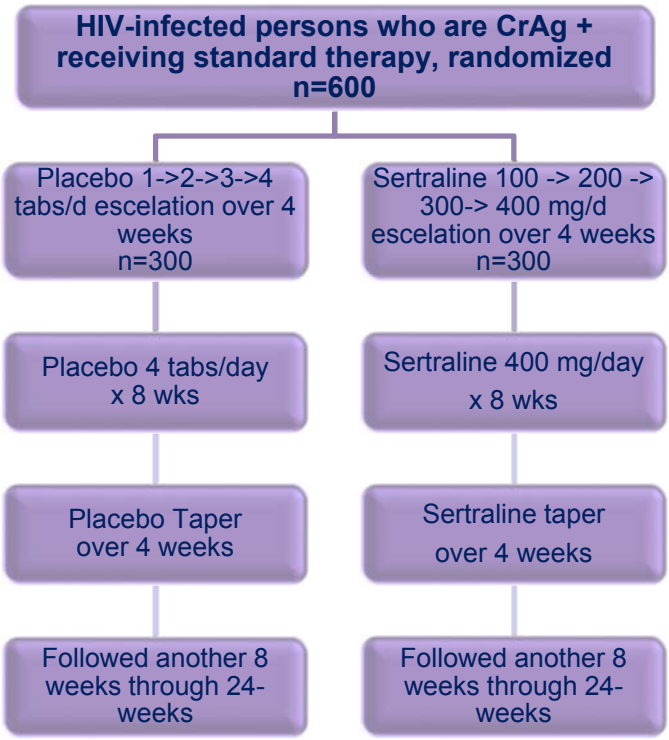
The purpose of the monitoring portion of the SMSAP is to:

1) Protect and ensure the safety of the subjects;
2) Ensure the validity and integrity of the data for the clinical trial;
3) Ensure that the clinical trial is monitored appropriately;
4) Ensure that the data collected can monitor safety and address protocol objectives;
5) Ensure that the executive committee and sponsor are aware of the schedule of monitoring for the clinical trial

The purpose of the statistical analysis portion of the SMSAP is to define *a priori* the analyses that will be completed for the primary and secondary endpoints, including the specification of pre-defined subgroups.

Study Overview

This Cryptococcal Antigen Screening plus Sertraline (C-ASSERT) Trial is a randomized trial comparing adjunctive sertraline versus placebo added to standard care for asymptomatic CrAg+ patients in Uganda . Study entry will occur within 1 week of initiating fluconazole antifungal treatment. Subjects will be randomized in a 1:1 allocation ratio to adjunctive placebo or sertraline. The adjunctive sertraline to be used in this trial is dosed at 100-200mg/day for two weeks, then 300-400mg/day for two weeks as induction therapy, followed by 400mg/day for 8 weeks, and then tapered over 4 weeks.



The primary endpoint is improved 6-month meningitis-free survival in CrAg+ HIV-infected persons using adjunctive sertraline when compared to standard therapy alone. Secondary endpoints include comparisons of the following outcomes across trial arms:

1. 6-month Survival Time
2. Incidence of Symptomatic Cryptococcal Meningitis
3. Incidence of Clinical Grade 3,4,5, and/or Serious Adverse Events
4. Incidence of Grade 3-5 Laboratory Adverse Events
5. Incidence of premature study drug/placebo discontinuation
6. Prevalence of depression by PHQ-9 score

The study will enroll 600 participants over a ≈4 year period. Once enrolled, each participant will be followed for 18 weeks for the primary endpoint, which allows for appropriate follow-up time for all secondary endpoints. Participants with a prior history of cryptococcal meningitis will not be enrolled in this trial.

Summary of Progress and Monitoring Reports

Table 1. Type of Reports and their distribution

Reports	Prepared By:	Frequency	Study Team	Safety Committee	IRB	DSMB	DAIDS Medical Officer
Study Progress Reports	Data Manager	Monthly					
Clinical Quality Management Report	Study Coordinator/ Data Manager	Quarterly					
Unexpected Serious Adverse Event Reports	Site PI	As needed <72 hours					
Early Safety Independent Review	Statistician	After every 40 subjects					
Sertraline-related or unexpected SAEs	Statistician	< 7 days					
New Pregnancy	Site PI	As Needed < 48 hours					
Open DSMB Report	Statistician	At least Annual					
Closed DSMB Report	Statistician	At least Annual					

The Trial Safety Committee consists of Drs. Jason Baker, Noeline Nakasujja, and Tihana Bicanic, and they will review after every 40 subjects have accrued 3 months follow up data until the DSMB assumes responsibility for oversight. Refer to Protocol Section 12.7.5.

The site biostatistician preparing the Progress, Monitoring and DSMB reports is Kathy Huppler Hullsiek PhD in conjunction with the Data Manager, Ms. Ananta Bangdiwala. DSMB reports are scheduled to be prepared after each ~25% of the trial population is enrolled, and at least annually.

Study Progress Reports

Study Progress Reports include reports on accrual, adherence to study protocol, baseline characteristics, and data completeness at each study site. Reports are pooled across study groups.

Purpose: The purpose of the Study Progress Report is to monitor enrollment to ensure that accrual goals are met in a timely manner, to inform sites of the number of subjects enrolled, and to inform sites of whether accrual is meeting specified target goals for each study site. The purpose of the Delinquency Report is to ensure a current database and to make Sites, Site PI, and trial PI aware of specific problems regarding missing clinical data for rapid resolution.

Responsibility for Preparation: Data Manager / Statistician

Frequency of Preparation: Monthly

Distribution The Study Progress Report is distributed as follows:

- Study Team
- NIH NIAID Representative

Contents: The contents of the Study Progress Reports are as follows:

Table 1: Participant Accrual

Table 2: Follow-up Status for Enrolled Participants

Table 3: Participant Baseline Characteristics

Table 4: Study Drug Administration Timing and Proper Randomization

Table 5: Summary of Grade 3, 4, and Grade 5 Adverse Events and Serious AEs

Table 6: Line Listing of Cumulative Clinical Adverse Events by Participant ID

Table 7: Summary of Laboratory Abnormalities by Visit

18.1. Accrual Report

The following reports will be provided:

- Enrollment by month and study site
- Cumulative enrollment versus goal, by study site
- Cumulative enrollment, overall

Line listing of study PID number with their randomization assignment is not proposed to assure that blinding is maintained and that the randomization sequence is not deciphered.

18.2. Follow-up for Enrolled Participants

Summary of participants currently:

- Receiving induction therapy (1-4 weeks)
- Receiving consolidation therapy (5-12 weeks)
- Receiving maintenance therapy (13-24 weeks)
- Terminated study

18.3. Baseline Characteristics Report

- The following baseline data will be cumulatively summarized:
    - Age
    - Sex
    - Baseline laboratory values
        - $CD4^+$ counts
        - Creatinine
        - Potassium
        - Total hemoglobin
        - ALT
        - Total bilirubin
    - Antiretroviral therapy (ART) status
    - Tuberculosis medication status
    - PHQ-9 depression score (Q9) and total score

18.4. Study Drug Administration Timing and Patient Care Indicators

Purpose: The Study Drug Administration Timing and Patient Care Indicators Report will monitor the timeliness of study and standard drug regiments relative to time of CrAg testing. It also monitors timeliness of diagnosis and enrollment in the trial relative to hospital admission.

The following indicators will be summarized by site:

- Time from CrAg+ to Sertraline
- Time from Informed Consent to Enrollment

18.5. Adverse Event

Adverse Events (AEs) will be summarized by;

- Number of new and cumulative number of AEs overall by Grade 3-5.
- Serious AEs

18.6. Cumulative Clinical Adverse Events

- Line listing by Participant ID

18.7. Summary of Laboratory Abnormalities

- By laboratory abnormality by visit and by DAIDS Grading

Clinical Quality Management Report (CQMP)

Purpose: To assure regulatory compliance and ongoing quality control and quality assurance of the clinical data collection.

Activities: Refer to CQMP for all details, in brief, activities will involve:

- Quality Control of CRF data for entry criteria, protocol adherence, and Good Clinical Practice (GCP) adherence
- Quality Assurance of data recorded
- Regulatory review

Responsibility for Preparation: Study Coordinators and Data Manager

Frequency: 1) Continuous activities
2) Quarterly summary reports

Contents (expanded from the monthly Progress Reports):

- Table 8: Line Listing of Protocol Violations by Participant ID
- Table 9: Summary of Data Completeness by Visit
- Table 10: Case Report Form (CRF) Delinquency Report
- Table 11: Delinquent CRFs by Participant ID

18.8. Protocol Violations

Purpose: The purpose of the Protocol Adherence Reports is to identify on an ongoing basis the adherence to the protocol and the incidence of protocol violations. The following reports will be provided:

- Consent withdrawn
- Inclusion criteria violations
- Exclusion criteria violations
- Line listing of PID by protocol violation

18.9. Delinquency Report

The Delinquency Report will include:

- Count of missing CRFs by study site, which are:
    - 4 – 8 weeks overdue
    - >8 – 12 weeks overdue
    - >12 weeks overdue
- Delinquency Summary by PID and site, which will include:
    - Number/type of delinquent CRF
    - Expected visit number of delinquent CRFs

Unexpected Serious Adverse Event Reporting

Purpose: To comply with national guidelines, all serious ***unexpected*** adverse events must be reported to the IRB within 3 working days. An adverse event related to cryptococcal meningitis (a pre-existing condition) will not be considered to be an unexpected event. *Expected* Serious Adverse Events (SAEs) will be reported at the frequency requested by the local IRB of record.

Refer to Protocol Section 10 on Adverse Event Reporting, and the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events (version 2014) and Adverse Event CRF.

Responsibility for Preparation: Site PI

Frequency of Reporting: As serious unexpected adverse events occur

The site PI will report serious unexpected adverse events to the IRB, Trial Safety Committee, and DAIDS medical officer within 72 hours of the awareness of the event

The Trial Safety Committee will review each unexpected SAE and perform an early independent review of trial safety (mortality + SAE incidence) after 40 subjects have been enrolled and accrued 3 months follow up. They will adjudicate any potentially sertraline-related AEs or unexpected SAEs.

Pregnancy Reporting

In the event that a subject becomes pregnant after enrolling in the study, she must be referred to the local antenatal clinic. ART will be guided per national guidelines in the antenatal clinic.

All pregnancies that occur during the study must be reported to the executive committee within 48 hours, regarding determination of continued fluconazole usage. Fluconazole is a known potential teratogen. Dependent on the duration of antifungal therapy and consultation with cryptococcal expert consultants (Drs. J. Perfect and T. Harrison), a recommendation will be made for the pregnant patient on the risks/benefits of fluconazole continuation, discontinuation, or dose reduction. This will be a non-binding recommendation. The decision to continue/discontinue/dose reduce fluconazole will be the exclusive choice of the research participant. The pregnant subjects may remain in the study regardless of their choice.

A review of fluconazole teratogenicity of is available at: drugsafetysite.com/fluconazole. All pregnancies should be reported to the Antiretroviral Pregnancy Registry by fax at +44 1895

825 005. More information is available at www.apregistry.com.

DSMB Reporting

18.10. Open Report to the DSMB

- Study progress and baseline data will be reviewed after each approximately 25% of subjects are randomized, and at least annually with any additional specified times as requested by the NIH DSMB.
- Open DSMB reports will be prepared for the overall randomized group and will include:
- Study progress reports: accrual, completeness of study follow-up, adherence to study protocol, data completeness
- Baseline data: participant characteristics, medical history, laboratory measures
- Baseline treatment status and regimens for CrAg + and HIV treatment
- Safety data (Grade 3-5 adverse events)

18.11. Closed Report to the DSMB

Closed reports will report by treatment group. The closed reports will include:

- Tables from the Open Report (by treatment arms) and Safety data (Grade 3-5 adverse events)
- Primary and secondary outcomes, including:
    - 6-month cryptococcal-free survival
    - incidence of symptomatic cryptococcal meningitis
    - incidence of adverse events
    - incidence of laboratory adverse events
    - incidence of premature study drug/placebo discontinuation
- Other noteworthy significant clinical events

Treatment groups will be formally statistically tested for:

- 6-month cryptococcal-free survival

Early Stopping Guidelines

A Lan-DeMets spending function analog of the O'Brien-Fleming boundaries will be provided at each DSMB report for the stopping guidelines for 6-month cryptococcal-free survival outcome. The O'Brien-Fleming boundaries will be truncated at alpha=0.002 (|Z|>3.09). The provided table assumes three interim analyses, and a final analysis with an overall two-sided cumulative alpha=0.05.

Interim Analysis	Sample Size	| Z |	P-value	Cumulative Alpha
1	~150 (25%)	> 3.09	0.002	0.0020
2	~300 (50%)	> 3.09	0.002	0.0037
3	~450 (75%)	> 2.38	0.016	0.0193
Final	~600 (100%)	> 2.02	0.031	0.0500

The study was designed assuming 25% and 16% with a primary event in the control and treatment groups, respectively. At the first DSMB review, the stopping boundary is unlikely to be crossed: if the event rate in the control and treatment groups was 25% and 37%, respectively, then the Z-statistic would be 3.2 and the stopping boundary would have been crossed. Thus for a consistent 25% event rate in the control arm, the event rate would need to increase from 16% to 32% in the treatment arm to stop the study at the first DSMB. The difference necessary to trigger early stopping will converge toward an approximate 9% absolute mortality difference by study conclusion.

The purpose of the early DSMB reviews is to assess the trends for safety and efficacy and allow for the DSMB to determine if more frequent than annual DSMB reviews are appropriate. If more interim analyses are desired by the DMSB, the Lan-DeMets spending function will be recalculated using O'Brien-Fleming boundaries and provided with each closed report.

We recommend that the DSMB consider early termination or protocol modification only when the O'Brien-Fleming boundary is crossed for the difference in 6-month cryptococcal-free survival.

Should a stopping boundary be crossed, we would recommend a sub-group analysis to determine whether the entire study should be stopped or a pre-specified subgroup excluded from further enrollment. Subgroups to consider include clinical site, ART use at baseline, and TB-status.

18.12. Sample Size Re-estimation

When approximately 300 participants (50%) have been randomized, a formal sample size re-

estimation will be conducted to assess whether the initial assumptions for 6-month cryptococcal meningitis-free survival were correct. This sample size re-estimation will be included as part of the closed report to the DSMB. The sample size re-estimation will be based on the pooled (and blinded) event rate and the 9% absolute difference in mortality expected between the groups. The sample size re-estimation will not take into account the interim treatment effect.

Potential options for the DSMB to recommend may include:

- Continuing the study as planned
- Increasing the sample size, if the interim-observed treatment effect size is smaller than had been anticipated but is still clinically relevant.
- Closing the study to enrollment.

18.13. Futility Analysis

The expected accrual rate is 125-150 participants per year. Enrollment in year 1 will likely be lower due to staggered starting of clinical sites, each of which is dependent on Funder permission to initiate.

At the second DSMB if the accrual is less than 75% of what is expected, the DSMB committee may ask the study team to submit a formal plan to increase enrollment.

If the conditional power is <25% at the time of any interim analysis, discontinuation may be considered as a possible recommendation by the DSMB. The DSMB will be given conditional power under both the design and the current data for their review. Ultimately, the DSMB will make their own decision, irrespective of any stopping guidelines.

Stopping for futility is always a possible DSMB recommendation.

Statistical Analysis Plan

For the primary and secondary outcomes one comparison will be done:

- Sertraline versus placebo

All analyses will be performed as intention-to-treat. Persons lost to follow up will be considered failures. All participants will be censored at 6 months.

Null Hypothesis: H_0: Sertraline has no survival benefit

Alternative Hypothesis: H_1: Sertraline has a survival benefit

6-month cryptococcal-free survival, the primary outcome, will be statistically tested via a Log-rank test with an indicator for treatment arm. Time to event analyses will also be summarized using Kaplan-Meier curves covering the time from enrollment to 6 months.

Secondary endpoints that can be analyzed with time-to-event methods (incidence of symptomatic cryptococcal meningitis, incidence of adverse events, incidence of laboratory adverse events, incidence of premature study drug/placebo discontinuation) will be summarized with cumulative incidence (to account for competing risk of mortality).[1]

- Symptomatic meningoencephalitis will be defined as meningitis symptoms plus:
    1) *Cryptococcus* culture positive meningitis,
    2) CSF CrAg+, or
    3) Cryptococcoma(s) by neuroimaging or post mortem exam.

The secondary outcome of prevalence of depression as per PHQ-9 questionnaire will be tested using a mixed effects regression model with a random intercept for individual to account for the intra-subject correlation induced by repeated PHQ-9 measures over time. An interaction indicator variable of time and treatment groups will assess differences in PHQ-9 depression scores. Additionally, we will summarize the prevalence of depression (by severity) from baseline through 12 weeks by randomized group.PHQ-9 Total Score Depression

Baseline demographic features of each study arm will be summarized, with statistical testing as appropriate for nominal and continuous variables to assure adequacy of randomization. If baseline variables differ between randomization groups, consideration of adjustment for such variable will occur.

Baseline characteristics known to be associated with developing cryptococcal meningitis (e.g. CrAg titer) and baseline characteristics known to be associated with mortality (e.g. CD4, anemia) among those who are CrAg + will be queried as to whether they are equally distributed among the two randomized treatment arms. If these are differentially distributed (not expected), a multivariable analysis will be conducted adjusting for the differentially distributed variables.

References

1. Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. Ann Stat 1998;16:1141–54.

Appendix C: Current Study Site List and Process Overview

Appendix C is a separate document from the protocol and will be updated so as to remain current.

C-ASSERT Trial Clinical Sites:

1) Infectious Disease Institute (IDI), P.O. Box 22418, Kampala, Uganda.
   Contact: Dr. Elizabeth Nalintya enalintya@yahoo.com
2) Joint Clinic Research Center (JCRC) – Mbarara Regional Centre of Excellence, Mbarara University of Science and Technology, 1 Hospital Road, Mbarara, Uganda
   Dr. Conrad Muzoora conradmuzoora@gmail.com
3) Medical Research Council (MRC) / Uganda Virus Research Institute (UVRI) Masaka Field Station, P.O. Box 1603, Masaka, Uganda
   Dr. Freddie Kibengo freddie.kibengo@mrcuganda.org

Overview of Laboratory Testing:

CRAG Screening: CrAg screening will be performed as per standard of care in accordance with national and WHO guidelines. In general, this screening will be predominantly performed as a lab-based reflex test at time of CD4 testing, whenever the CD4 count is <100 cells/μL. This will be performed at the local site CD4 referral laboratory. These CD4 laboratories are on-site for clinics of: IDI-Kampala, TASO-Kampala, MRC-Masaka, and JCRC-Mbarara. In Kampala, the KCCA network of primary care clinics send CD4s to MUJHU-affiliated laboratory. Additional outside clinics may also send CD4 specimens to TASO, MRC, and JCRC laboratories. Ugandan guidelines also recommend CrAg testing among individuals with suspected ART failure or WHO clinical stage III/IV illness, irrespective of the CD4 count.

The initial CrAg positive plasma specimen tested at any non-DAIDS approved laboratory shall be transported for verification of CrAg-positivity at a DAIDS-approved laboratory (e.g. MU-JHU lab) or repeated at time of enrollment. Hepatic ALT (exclusion criterion) may be run on collected serum or plasma specimens collected at this time point at the DAIDS-approved laboratory.

Pre-Entry: Will assess for trial eligibility. Pregnancy testing for women of child-bearing potential will be tested at the local site laboratory on urine or serum. Pregnancy testing is required within 14 days of enrollment for potentially childbearing women. If the exclusion ALT laboratory was not able to be run on a specimen collected at screening (i.e. insufficient volume), ALT must be run prior to randomization. The ALT can be run via 'stat' sample from the local laboratory or via a Piccolo point-of-care chemistry instrument with a FDA-approved, CLIA waived panel. ALT testing is required within 30 days of enrollment.

If plasma was not stored from the screening visit (e.g. insufficient volume), CrAg status will be verified on a pre-entry specimen at a DAIDS-approved facility. This CrAg testing may be by fingerstick or blood draw. Any external lab result from a non-DAIDS approved facility will need to be repeated for verification. Only the DAIDS-approved lab facility lab result is official.

Enrollment: Immediately after the pre-entry visit (i.e. same day), persons who are eligible will be randomized. Enrollment visit blood draw will occur. Additional pre-ART laboratories may be drawn at this visit for purposes of routine HIV care in accordance with local clinic policy (e.g. hepatitis B screening, creatinine, hemoglobin). Additional leftover serum and plasma shall be stored at -80ºC for future pathogenesis studies. PBMCs may be collected in Kampala depending on the time of day of the clinic visit. PAXGene RNA tubes may be collected at sites, based on financial resources available.

Cerebrospinal Fluid (CSF) Testing: Persons with suspected meningitis shall be referred for a diagnostic lumbar puncture (LP). Such LPs may occur in the clinic, referral center, or hospital as appropriate. CSF specimens shall be tested locally for CSF CrAg and routine CSF profile. CSF cultures shall be performed at the Makerere University Clinical Microbiology laboratory or Mbarara University Microbiology laboratory. Specimens from Masaka shall be sent to Kampala. Refer to quantitative CSF culture SOP for methodology. All collected CSF shall be stored and cryopreserved at -80ºC.

A participant symptomatic for meningitis with a positive CSF CrAg or CSF culture = cryptococcal meningitis primary endpoint. CSF CrAg is more sensitive than culture (e.g. sensitivity >99% CrAg LFA vs. ~94% CSF culture not on ART, ~85% CSF culture when on ART).

Follow Up Visits:

There is routine laboratory monitoring specified by the protocol at 4, 8, and 12 weeks although the safety profile of sertraline (and fluconazole) are excellent. In the event of a serious adverse event, relevant specimens should be collected as appropriate by physician discretion. For example, in the event of jaundice, collection of liver function tests (e.g. AST, ALT, total bilirubin) would be appropriate. Such specimens may be run locally for purposes of immediate clinical care. However, all specimens for a serious adverse event (SAE) shall be stored and transported to a DAIDS-approved lab for confirmatory testing. The laboratory values at the DAIDS-approved laboratory shall be used for official study-related documentation.

Total Test Volume of Protocol Specified Tests

Test	N	Volume (mL)	Total Volume (mL)
CBC	4	4-6	16-24
Electrolytes, Creatinine	4	4	16
Liver Function Tests	5	4	20
Total			**52-60**

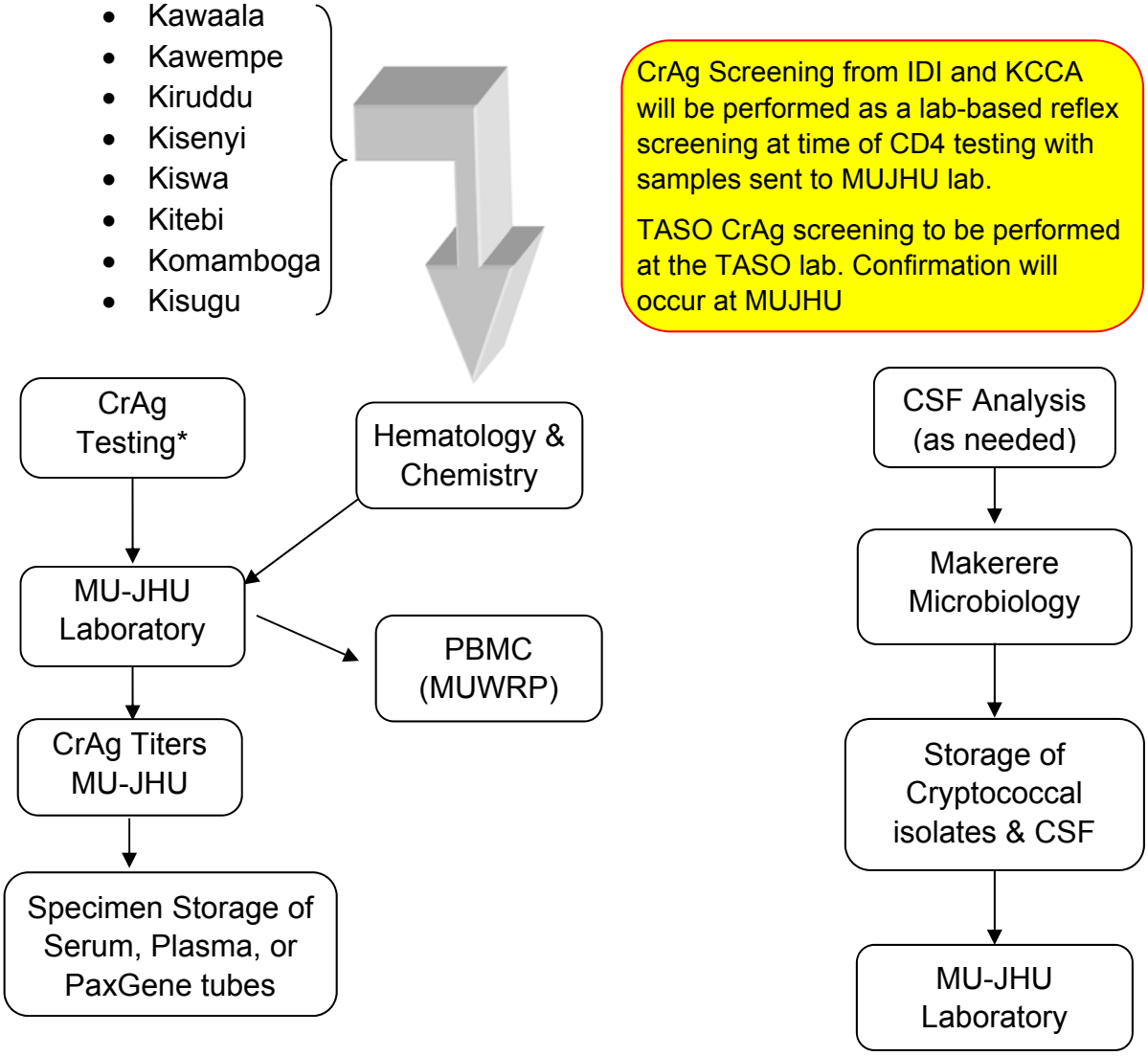
Pharmacy Overview:

This is a randomized, double-blind placebo controlled trial. Study medicine (sertraline or matched placebo) will be prepared at the central pharmacy at the IDI in Kampala. The pharmacist of record is Eva Laker. Study medicine bottles will be labeled with a unique identifier. Biostatisticians at UMN will generate labels for the pill bottles. Each label will be pre-printed with only a unique drug ID number. There will be two sets of labels for Sertraline or Placebo. The labels will be delivered to the pharmacy staff at IDI in Kampala. These unique identifiers will be in random sequences, such that there is no numeric pattern to identify sertraline or placebo bottles. In Kampala, medicines will be dispensed from the IDI pharmacy. In Masaka and Mbarara, a small supply will be provided from the IDI pharmacy (e.g. sufficient for ~20 participants) and replenished as needed from the satellite pharmacies.

KAMPALA SITE OVERVIEW

1) Infectious Diseases Institute (IDI) / College of Health Sciences, Makerere University, Kampala, Uganda for enrolment and follow up with referrals from health facilities including:

   - The AIDS Support Organisation (TASO), Kampala
   - Kampala Capital City Authority (KCCA) Clinics, Kampala, Uganda
     - Kawaala
     - Kawempe
     - Kiruddu
     - Kisenyi
     - Kiswa
     - Kitebi
     - Komamboga
     - Kisugu

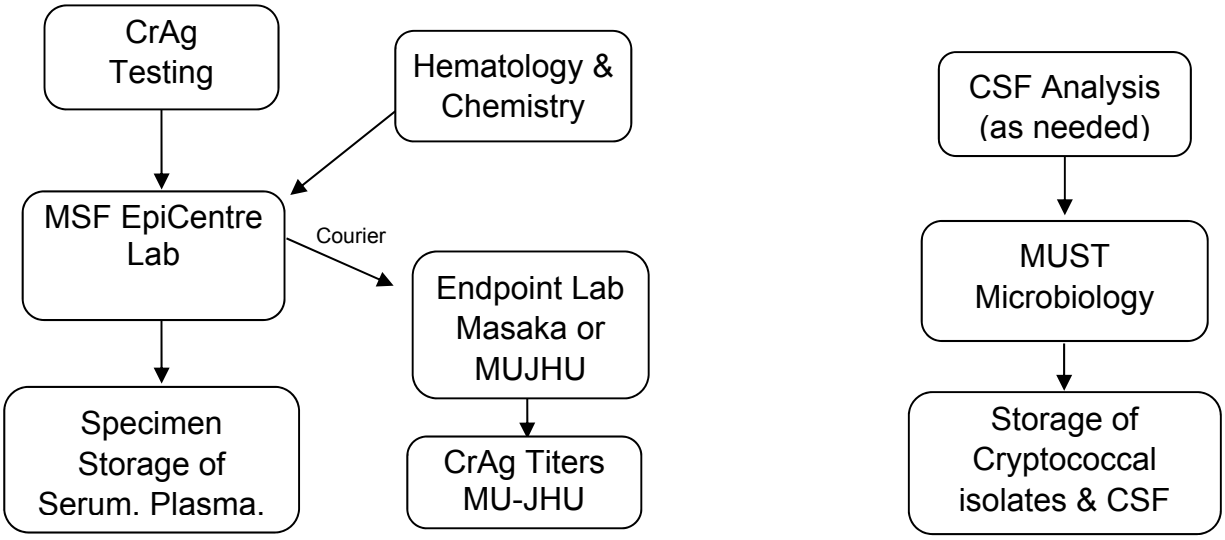


IDI Pharmacy will be the pharmacy of record for the C-ASSERT trial with Eva Laker as pharmacist of record. IDI pharmacy will perform the dispensing for all Kampala sites. With couriers displacing medicines to KCCA study sites.

* CrAg LFA testing for TASO clinic will initially be performed at TASO. All follow up research-related labs performed at MUJHU.

MBARARA SITE - OVERVIEW OF LABORATORY TESTS

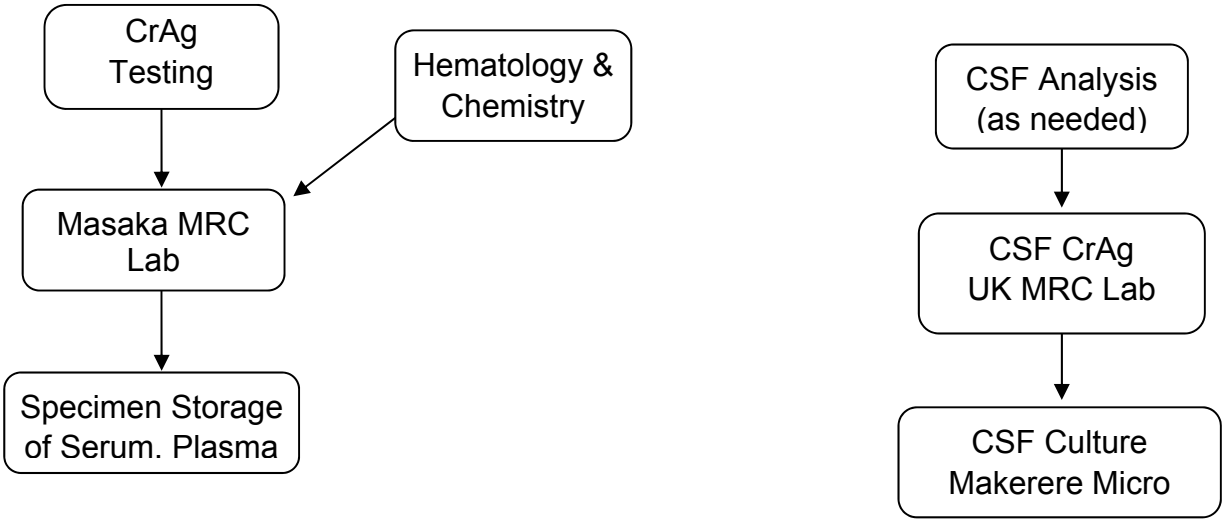
Lab-based reflex RAG screening will be implemented at local CD4 labs (e.g. MSF Epicentre). Outside clinics may refer patients with the CrAg testing repeated at the study site.



Mbarara Satellite Pharmacy: Will maintain a small stock of blinded study medication as prepared by the IDI pharmacy in Kampala for local dispensing.

MASAKA SITE OVERVIEW

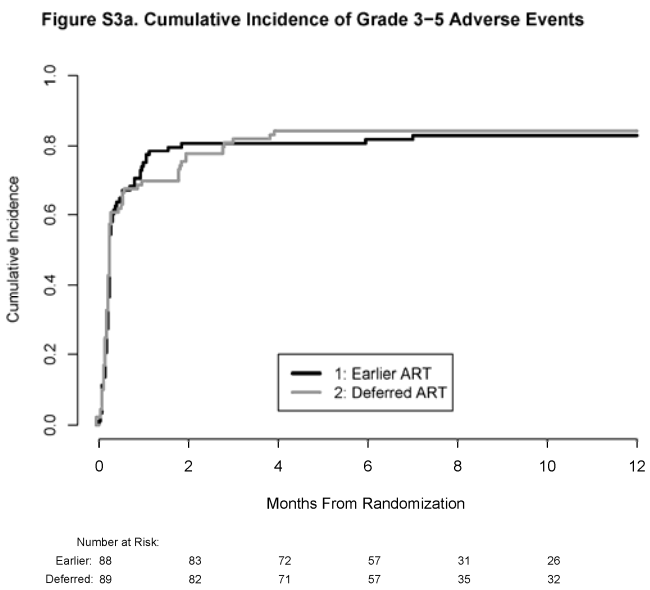
Outside clinics may perform CrAg testing with referral of potential research participants to MRC-Masaka, where repeat CrAg testing will be performed. The MRC-Masaka laboratory is GCLP compliance and participates in EQA.



MRC-Masaka Satellite Pharmacy: Will maintain a small stock of blinded study medication as prepared by the IDI pharmacy in Kampala for local dispensing.

Appendix D. Adverse Events observed in COAT and ASTRO-CM sertraline Trial.

Cryptococcal Optimal ART Timing (COAT) Trial in Uganda used amphotericin and fluconazole with a randomized timing of ART initiation. No sertraline was given. Data below are as published in the manuscript's supplemental appendix.[35] In total, 436 Grade 3-5 adverse events occurred in 177 subjects with cryptococcal meningitis. The majority were asymptomatic lab abnormalities, related to AIDS, cryptococcosis, or amphotericin therapy.



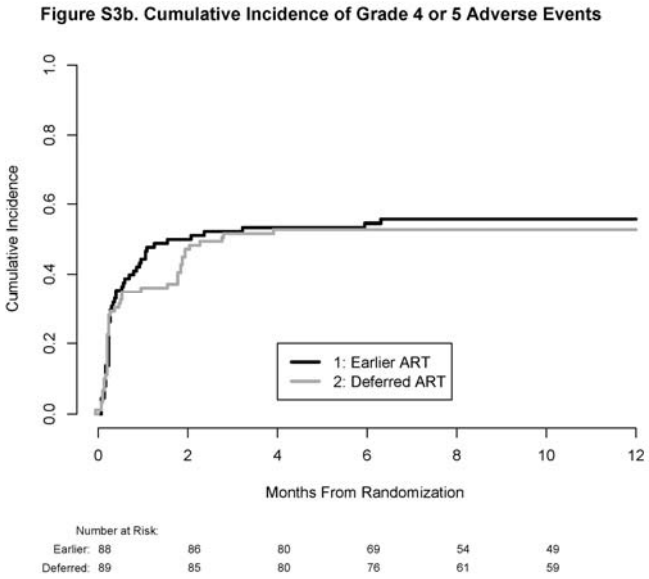


Figure S3a displays the cumulative incidence of time to a first Grade 3-5 adverse event by trial arm as defined by the NIAID DAIDS Toxicity Grading Scale, 2009. The AE distribution by body system was:

Distribution by Body System	Earlier ART	Deferred ART
Hematology	99 (47.8%)	128 (55.9%)
Chemistries	65 (31.4%)	53 (23.1%)
Infection	13 (6.3%)	26 (11.4%)
Neurological	11 (5.3%)	5 (2.2%)
Gastrointestinal	6 (2.9%)	8 (3.5%)
Cardiovascular	6 (2.9%)	5 (2.2%)
Systemic	4 (1.9%)	2 (0.9%)
Skin, dermatological	2 (1.0%)	1 (0.4%)
Respiratory	1 (0.5%)	1 (0.4%)
Overall (Number of events)	207 (100%)	229 (100%)

The most commons adverse events included anemia (n=88), neutropenia (n=54), leukopenia (n=24), hyponatremia (n=21), hypokalemia (n=23), and elevated creatinine (n=18).

Grade 5 events occurred in 14 (15.9%) persons randomized to earlier ART and 11 (12.3%) randomized to deferred ART. Grade 5 events did not include deaths from *Cryptococcus*.

Randomized ASTRO-CM Trial Participants Adverse Events:

This reports on the ongoing Grade 4, 5, and SAEs observed during the randomized trial for cryptococcal meningitis with amphotericin + fluconazole 800 mg/day +/- sertraline 400mg/day. As of May 6, 2017, n=427 randomized participants. There has been no statistical difference in the incidence of adverse events. **There have been no sertraline-related Grade 4-5 AEs or SAEs among n=218 participants randomized to 400mg/day of sertraline.**

Table A1	Placebo	Sertraline	P-value[2]
Number Randomized	209	218	
Number of AEs per person, N (%)			0.26
None	165 (78.9%)	176 (80.7%)	
One	32 (15.3%)	37 (17.0%)	
Two	10 (4.8%)	5 (2.3%)	
Three or more	2 (1.0%)	0 (0.0%)	
Overall	209 (100.0%)	218 (100.0%)	
Number of AEs by initial grade, N (%)			0.60
Grade 4 AE	50 (86.2%)	38 (80.9%)	
Grade 5 AE	8 (13.8%)	9 (19.1%)	
Total	58 (100.0%)	47 (100.0%)	
Number of AEs by final grade, N (%)			0.77
Grade 3 AE	8 (13.8%)	8 (17.0%)	
Grade 4 AE	29 (50.0%)	20 (42.6%)	
Grade 5 AE	20 (34.5%)	19 (40.4%)	
Unknown (ongoing follow up)	1 (1.7%)	0 (0.0%)	
Total	58 (100.0%)	47 (100.0%)	

Grade 3 = Severe, Grade 4 = Life Threatening, Grade 5 = Non-CM related Death.

Table A2. Adverse Events - Relations and Outcomes

	Placebo	Sertraline	P-value
Number Randomized	209	218	
Relationships[1], N (%)			
To Sertraline	0 (0.0%)	0 (0.0%)	
To Cryptococcus	22 (37.9%)	21 (45.7%)	0.55
To Antifungal therapy	42 (72.4%)	28 (60.9%)	0.29
To Antiretroviral drugs	18 (31.0%)	10 (21.7%)	0.37
To HIV/AIDS	46 (79.3%)	38 (82.6%)	0.80
Outcome, N (%)			0.32
Recovered/resolved	26 (44.8%)	19 (41.3%)	
Recovered/resolved with sequelae	0 (0.0%)	3 (6.5%)	
Severity worsened to Grade 5	12 (20.7%)	12 (26.1%)	
Death, unrelated	16 (27.6%)	10 (21.7%)	
Chronic, not expected to resolve	0 (0.0%)	0 (0.0%)	
Persistent, expected to resolve	2 (3.4%)	2 (4.3%)	
Unknown	2 (3.4%)	0 (0.0%)	
OVERALL	1 (1.7%)	47 (102.2%)	

[1]The relationships are NOT mutually exclusive. Multiple relationships could be recorded. Relationships could be possible, probable, or definite.

Below is a line listing of Grade 4, 5, and SAEs. The majority of AEs have been related to sequelae of AIDS, cryptococcal meningitis, and/or amphotericin B deoxycholate. None have been deemed as an AE “related” to sertraline, in the opinion of the investigator.

Table A3. Types of Adverse Events by DAIDS Toxicity Table Category

	Placebo	Sertraline
Number Randomized	209	218
Fever	0	4
Infection	1	4
Localized injection site reaction	0	1
Hypotension	1	1
Thrombosis/embolism	0	1
Alteration in personality	1	1
Headache	1	1
Seizure (new onset)	2	2
Dyspnea or respiratory distress	4	1
Hemoglobin <8.5 g/L	27	17
Decreased platelets <50 $x10^9$/L	1	0
Acidosis	2	0
ALT (SGPT), high	0	2
Decreased serum bicarbonate	1	0
Bilirubin (total), high	0	1
Creatinine, high	6	3
Potassium, high	0	2
Potassium, low	6	2
Sodium, low	1	1
Miscellaneous	4	2