Appendix B: Study Monitoring and Statistical Analysis Plan

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1. 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:

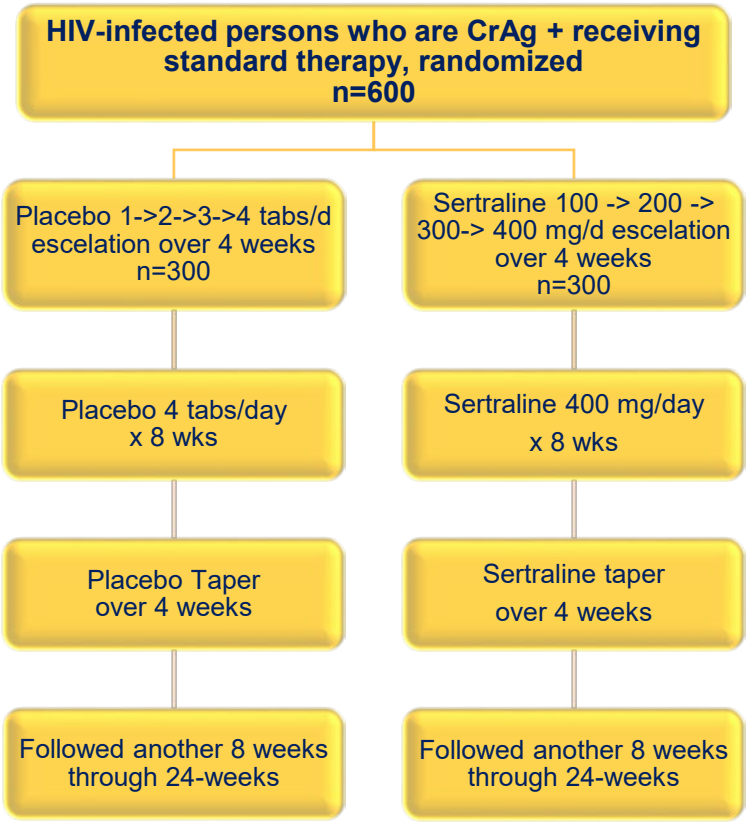
1) Protect and ensure the safety of the subjects;
2) Ensure the validity and integrity of the data for the clinical trial;
3) Ensure that the clinical trial is monitored appropriately;
4) Ensure that the data collected can monitor safety and address protocol objectives;

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.

2. 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.

3. 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.

4. 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

4.1 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.

4.2 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

4.3 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

4.4 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

4.5 Adverse Event

Adverse Events (AEs) will be summarized by;

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

4.6 Cumulative Clinical Adverse Events

- Line listing by Participant ID

4.7 Summary of Laboratory Abnormalities

- By laboratory abnormality by visit and by DAIDS Grading

5. 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

5.1 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

5.2 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

6. 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.

7. 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.

8. DSMB Reporting

8.1 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)

8.2 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

9. 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.

9.1 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.

9.2 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.

10. 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.

11. 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.