Author's response to reviews

Title: AMBITION-cm. Intermittent High Dose AmBisome on a High Dose Fluconazole Backbone for Cryptococcal Meningitis Induction Therapy in sub-Saharan Africa: Study Protocol for a Randomized Controlled Trial

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Author's response to reviews: see over
The Editors-in-Chief

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Dear Editors,

Title of paper: AMBITION-cm. Intermittent High Dose AmBisome on a High Dose Fluconazole Backbone for Cryptococcal Meningitis Induction Therapy in sub-Saharan Africa: Study Protocol for a Randomized Controlled Trial: MS 1623230334152820

Many thanks for your e-mail of the 19\textsuperscript{th} May regarding the above manuscript and for forwarding the comments of the reviewer. We are pleased that this paper was found to be of interest and that submission of a modified version of the manuscript has been requested. We have now revised the paper in light of the reviewer's comments, and feel it has been strengthened in the process. The modifications have been approved by all authors. Our point by point responses to the reviewer's comments are detailed below (page and line numbers refer to the revised manuscript):
Response to the reviewer’s comments

We thank the reviewer for his positive and constructive reviews. Responses to each specific comment are listed below.

Major compulsory revisions

Comment 1. “No details regarding the statistical analyses planned for the study are provided. At a minimum, can the authors please identify the main analysis for the primary outcome (Step 1 and Step 2), including analysis methods for statistical comparisons. Can the authors also provide information on effect measures and significance levels. Finally, the authors should also detail how missing data will be handled and any subgroup analyses that are planned.”

Response 1. For Step 1-Linear regression models will be estimated. Mean rate of decrease in CSF cryptococcal CFU (early fungicidal activity) will be the dependent variable and treatment group (using the standard treatment arm as a comparator) will be the primary independent variable. The comparator groups will be compared to the standard treatment arm for non-inferiority using standard criteria with an acceptable delta of 0.2 log10CFU/ml/day. Following crude analysis, adjustment will be made for baseline fungal burden, which has been found to be associated with rate of clearance in previous studies, and CD4 cell count, giving summary differences with 95% confidence intervals. As per the crude analysis, comparator groups will be compared to the standard treatment arm for non-inferiority using standard criteria with an acceptable delta of 0.2 log10CFU/ml/day. All other variables that are found to differ between the treatment arms will also be included as covariates.

For Step 2-The primary endpoint is mortality within the first 2 and 10 weeks by treatment arm. The short course Ambisome regimen chosen will be considered non inferior to the standard regimen if the lower bound of the 95 percent confidence interval for the difference between the two groups, those receiving the short course regimen minus those receiving the standard regimen for the primary end point (in the proportion of patients who have died by 2 and 10 weeks) is no lower than –15 percent. The two treatment groups will be compared with use of the Cochran–Mantel–Haenszel test for categorical data. In initial analysis patients lost to follow-up will be censored from the analysis. A sensitivity analysis will be performed in which all patients lost to follow-up are assumed to have died, along with a time to event analysis. A per-protocol analysis will also be performed. These changes have been incorporated into the revised manuscript (page 16, line 12).
Comment 2: "Primary outcome of step 1: In the outcomes section, can the authors clearly specify the time point at which the EFA measurement will be taken. Based on other sections in the protocol, it seems that it will be based on the second LP" 

Response 2: Cryptococcal clearance rates will be calculated using a summary statistic for each patient, the rate of decrease in log CFU per ml CSF per day derived from the slope of the linear regression of log CFU against time for each patient. Thus the measure is derived from all LPs performed, not just a single time point. A linear regression model will be used to compare mean rates of decline or early fungicidal activity (EFA) for each experimental treatment, giving summary differences with 95%CI and significance levels. We will adjust analyses for potential confounding factors, including baseline fungal load. This has been clarified in the revised manuscript (page 15, line 21).

Comment 3: “Recruitment: No details are provided regarding recruitment into the study. Can the authors clarify expected recruitment rates, estimates for eligible patients and the estimated duration of the recruitment period and any plans to monitor recruitment during the trial.”

Response 3: We anticipate the study to enroll 60 patients in Botswana and 160 patients in Tanzania over a 2 year period. This is feasible based upon historical numbers of admissions and previous studies in these sites. We have added this information to the manuscript (page 18, line 10).

Comment 4: “The study is funded by a Gilead investigator initiated award. Can the authors clarify the role of the sponsor in the study, if any.”

Response 4: The study is sponsored by St. George’s University of London. The trial is funded through an investigator initiated award from Gilead Sciences. The funders have had no role in the trial design, and will not be involved in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication. This has been made clear in the Acknowledgements and Funding section.
Minor essential revisions

Comment 1: “The authors clearly state the method of random sequence generation and allocation concealment. However, the allocation ratio was not specified although it seems to be 1:1. Can the authors add this information.”

Response 1: The allocation ratio is 1:1 and the information has been added to the revised version of the manuscript (page 14, line 2).

Comment 2: “The study is stated as being open-label and unblinded. Does this also mean that outcome assessors are unblinded? It would be helpful if the authors can specify this detail.”

Response 2: The clinical team members are not blinded to patients’ randomization arm, however the microbiologists performing CFU analysis will be blinded. This information has been added to the manuscript on page 14, line 14.

The manuscript has not been published in this or a substantially similar form (in print or electronically, including on a web site), nor accepted for publication elsewhere, nor is it under consideration by another publication. There are no potential conflicts of interests to report. I have signed this letter as confirmation that all authors have read and approved the paper, have met the criteria for authorship as established by the International Committee of Medical Journal Editors, believe that the paper represents honest work, and are able to verify the validity of the results reported.

I hope this manuscript is now suitable for publication in Trials Journal and I look forward to your feedback in due course.

Yours sincerely,
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(on behalf of all of the authors)