Mortality Reduction after Oral Azithromycin

Contingency Study, Niger, Year 3 and after

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Statistical Analysis Plan

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Introduction

This document (Statistical Analysis Plan, SAP) describes the planned analysis and reporting for the clinical trial, **Mortality Reduction after Oral Azithromycin**, Contingency Study, Years 3-4, Niger. It includes specifications for the statistical analyses and tables to be prepared for the interim and final Clinical Study Report. This study is a Phase IV clinical trial to compare methods to reduce childhood mortality using mass administration of azithromycin (Pfizer, CAS 83905-01-5) compared to placebo. The content of this Statistical Analysis Plan meets the requirements stated by the US Food and Drug Administration and conforms to the American Statistical Association's Ethical Guidelines.

The following documents were reviewed in preparation of this Statistical Analysis Plan:

- Mortality Reduction after Oral Azithromycin, Protocol
- Mortality Reduction after Oral Azithromycin, Statistical Analysis Plan
- Mortality Reduction after Oral Azithromycin, Manual of Operations
- ICH Guidance on Statistical Principles for Clinical Trials

The planned analyses described in this SAP will be included in future manuscripts. Exploratory analyses not necessarily identified in this Statistical Analysis Plan may be performed to support the analysis. Unplanned analyses not delineated in this Statistical Analysis Plan will be documented as such in the final Clinical Study Report and manuscripts.

This document will be reviewed prior to the enrollment of patients. All subsequent changes will be indicated by detailed change log in the Appendix.

MORDOR Stage 2 Contingency Study

1 Introduction

Should the primary analysis of azithromycin to prevent mortality reveal evidence of a benefit, we propose to proceed with two additional studies in Stage 2.

2 Design

Contingency study A. All communities in Niger will receive treatment at the conclusion of MORDOR Stage 1 (original study). Specifically, azithromycin communities will continue with azithromycin, and placebo communities will receive azithromycin at the 24 month visit (census number 5). Follow-up will occur at month 30 and month 36, providing one additional year of data.

Contingency study B. At the 36 month visit, rerandomization will occur, and half of communities will begin to receive placebo.

3 Analysis

The primary outcome is mortality, analyzed in the same way as in MORDOR Stage I, i.e., present on an initial census, and absent on the next follow-up census due to death. Since the study has been extended, the same analytic template will be used for each visit, e.g. at 48, 54, and 60 months. Each visit that is conducted will be reported.

Study A:

1. Sample size considerations.
    a. Assuming communities of size 668 with approximately 17% in the target age range, an effect size of 20%, 10% loss to follow-up per year, 2% mortality per year, and a CV of 0.51, 312 communities per arm provides approximately 87% power (see MORDOR Phase I SAP for details).
    b. Updating based on data from Phase I (Niger), we find the following. Assuming the observed CV of 0.34, a death rate of 2% per year results in approximately 80% power to detect a 17% effect. A death rate of 2.5% per year (and a CV of 0.34) result in approximately 80% power to detect a 15.5% effect size.
2. Primary prespecified analysis: We propose to compare the former placebo arms with the former azithromycin arms, using negative binomial regression. Significance testing is two-sided with an alpha of 0.05, and will be based on permutation testing. (This analysis is identical in every way to the country subgroup analyses conducted in Stage 1). This is restricted to the period from 24 months to 36 months, covering two six-month study intervals.

3. We propose, as a prespecified secondary analysis, a longitudinal comparison of the original placebo communities. We propose to contrast the first four study intervals during which these communities received placebo, to the last two study intervals during which these communities received azithromycin. Analysis will be conducted using negative binomial regression, with a permutation P-value reported (two-sided, alpha=0.05). The community-randomized nature of the trial is thus taken into account.
4. Additional supplementary analyses will be distinguished from the two prespecified analyses above. In particular, the following will be reported:
    a. Analysis of the seasonality and timing of deaths: time of death, time of treatment for those who died, and time since treatment for all deaths. We will conduct comparisons of time since treatment conditional on death based on the Cramer-von Mises test (exactly as in Stage 1), additionally comparing across treatment arms and longitudinally. Comparisons of equality of circular means will be used to assess seasonality of death and of treatment, comparing between randomization arms and longitudinally. We will, in particular, focus special attention and reporting to a comparison of phases 1-4 to 5-6 within the placebo arm.
    b. Analysis will be reported according to the same age classes used in the original study (Keenan et al, NEJM, 2018), i.e. 1-5, 6-11, 12-23, 24-59 months.
    c. We will examine the difference between phase 5 and 6 in the same way.
    d. We will report longitudinal analysis of mortality changes in the original azithromycin arm, using a linear predictor of study interval (1—6).
    e. We will compare estimated treatment effect at a regional level using placebo mortality rates as a predictor.

Study B (years two and three of mortality contingency study)

1. Sample size considerations are the same as in Study A (year 1).
    a. Assuming communities of size 668 with approximately 17% in the target age range, an effect size of 20%, 10% loss to follow-up per year, 2% mortality per year, and a CV of 0.51, 312 communities per arm provides approximately 87% power (see MORDOR Phase I SAP for details).
    b. Updating based on data from Phase I (Niger), we find the following. Assuming the observed CV of 0.34, a death rate of 2% per year results in approximately 80% power to detect a 17% effect. A death rate of 2.5% per year (and a CV of 0.34) result in approximately 80% power to detect a 15.5% effect size.
2. The primary prespecified analysis will contrast the rerandomized placebo arm with the rerandomized azithromycin arm. This will be conducted with negative binomial regression, using a two-sided alpha of 0.05 and a permutation test by randomization arm.
3. Supplementary analyses will control for prior history (original randomization arm).
4. Longitudinal analysis for communities who always received azithromycin will be reported.
5. We will compare all four groups for the last year of data (two histories by two final treatments).

6. Additional analyses by the same age groups as before and by phase will be reported.
7. Models of timing, seasonality, and geography will be reported as secondary analysis.

Morbidity Study. Analysis of morbidity communities will follow the exact template as Stage I. Primary prespecified analyses will be two-sided, at an alpha of 0.05. However, the morbidity communities are still receiving placebo. Note that we will report the full follow-up at 48 months separately from the Stage 1 primary analyses. We will also look for secular effects longitudinally over the four years of the full study, as well as phases 5-8 separately.

1. Anthropometry: we will examine height and weight longitudinally, and WFH Z score secondarily. Please see the Stage 1 SAP for details.
2. Other analyses will include drug resistance and malaria, as in Stage 1.
3. Note that we do not do nares or conjunctiva swabs, conjunctival photos, or any swabs on 7-12 year olds in the contingency study.

Statistical considerations

1. Communities which drop out of the study will be omitted from analysis for all study period for which we have no data for that community. If a community rejoins at a particular phase, it will be included as before.
2. Evidence of poor model fit to the negative binomial regression will justify reporting additional models.
3. Permutation tests will be conducted with 10000 replications. If the critical value 0.05 lies within the Monte Carlo confidence interval for the P-value, we will replicate with 10,000,000 replications, and this will be reported.

Resistance comparison.
160 total communities from the mortality contingency study B (above) will be randomized to the 4 arms (blocked on baseline assignment, but not on rerandomization), and specimens collected from each of 36 children randomly selected from the census. The children will be selected from the age range 1-59 months (inclusive), at the 54 month census. The following outcomes are to be assessed:

Rectal Swab samples:

1) Outcome: Normalized read number for macrolide resistance determinants from DNA sequencing. These are to be physically pooled by community prior to sequencing; individual specimens will not be analyzed separately, and individual-level analysis will not be possible.
   Analysis: We propose to use pairwise Wilcoxon rank-sum tests between each of the four treatment history groups, accounting for multiple comparisons as described [1-3]. All randomized communities will be included in the permutation testing.
   This is the primary analysis for this study. All other analyses for the rectal swab collection (below) are secondary.
   As a methodological sensitivity analysis, the Kruskal-Wallis (nonparametric one-way ANOVA), comparing all four arms. Such sensitivity analyses will be sharply distinguished from the primary prespecified analysis.

Mean fold difference between arms will be reported or plotted, as well.

2) Normalized read number of *Campylobacter* at the genus and species levels, derived from DNA sequencing. We will systematically compare the relative abundances of individual microbial taxa between groups at the species level by negative binomial Wald test using the Benjamini–Hochberg procedure for controlling FDR [4]. Differentially abundant taxa with a false discovery rate < 0.05 and a twofold change will be considered as notable. Sensitivity test will be perform by first using the two-sided Kruskal–Wallis test at a predefined alpha of 0.05. Significantly different vectors resulting from the comparison of relative abundances between any two groups will be used as input for linear discriminant analysis (LDA), which produces an effect size [5]. The LDA will be set at 2 for all analyses.
3) Beta lactam resistance determinants will be analyzed, as in 1) above.
4) Other analyses will be conducted using the same pooling method, but will feature the use of Benjamini-Hochberg FDR procedures. The FDR is prespecified at 0.05.
    a. Non-macrolide resistance determinants other than beta-lactam
    b. Occurrence of different viruses or bacteria (we exclude eukaryotic organisms). Of particular importance are *Chlamydia* spp., malaria, and tuberculosis.
5) As in 1) above, but with the following outcome for each community: bacterial species normalized read counts. Statistically, we propose PERMANOVA comparison of all 4 arms, based on the L_2 norm. As a sensitivity analysis, we will compare using the L_1 norm.
6) As in 4), but with species diversity as the primary outcome. We propose use of the L_2 norm for the primary analysis, and then, as a sensitivity analysis, we will compare using the L_1 norm, both expressed as effective number. The *P*-values will be derived from permutation.

Sample size. We chose N=160 total comunities, which provides approximately 80% power to detect a 3.5-fold difference in any pairwise comparison (assuming P=0.01, and an approximate SD of the base two logarithm of the read count (plus 1) of 2.25). The SD of the the log read count was derived from the placebo arm of the MORDOR 36 month data. The effect size (3.5 fold) is approximately half of what was observed in the MORDOR 36 month data.

NP Swab samples:

1) Analysis will compare macrolide resistance at the community level, using the Westfall-Young method [1] as above. This is the primary analysis for the NP swab study.

2) Exploratory analysis comparing the occurrence of different organisms at the genus and species levels. We prespecify an FDR of 0.05.

References

[1] Westfall PH, Troendle JF. Multiple testing with minimal assumptions. *Biometrical Journal* 50(5): 745–755, 2008.

[2] Ge Y, Dudoit S, Speed TP. Resampling-based multiple testing for microarray data analysis. Test 12: 1–77, 2003.
[3] Westfall PH, Young SS. *Resampling-Based Multiple Testing: Examples and Methods for p-Value Adjustment*. Wiley, New York, 1993.
[4] Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome biology 2014, 15:550.
[5] Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C: Metagenomic biomarker discovery and explanation. Genome biology 2011, 12:R60.