GAMIN

MORDOR Project

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Statistical Analysis Plan

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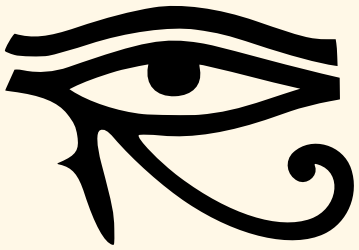

Introduction

This document (Statistical Analysis Plan, SAP) describes the planned analysis and reporting for the clinical trial, **GAMIN: Gut and Azithromycin Mechanisms in Infants and Neonates in Burkina Faso**. 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:

- Azithromycin for the reduction of child and infant mortality: Longitudinal Component, Manual of Operations and Procedures
- Azithromycin for the prevention of neonatal mortality in Burkina Faso: an individual-randomized trial, Statistical Analysis Plan
- Statistical Analysis Plan, MORDOR Trial
- 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. Note that unplanned analyses will be broadly grouped into two categories:

1. Sensitivity analyses for pre-specified outcomes
2. Other unspecified analyses

Sensitivity analyses are defined as alternate ways of modeling the primary outcome to ensure the finding was not dependent on the analytic plan, and these will not be subject to a multiple comparisons correction. Other unspecified analyses will be declared hypothesis generating or subject to an alpha level of 0.001.

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

Contents

1 Summary

1.1 Longitudinal Trial

The trial profile is given in the Manual of Operations. In the longitudinal trial, children are randomized to two arms: **Azithro** and **Control**. The trial is a placebo-controlled, double-masked (i.e., double-blind), individual-randomized clinical trial.

Objectives.

Determine the effect of a single dose of azithromycin for children aged 8 days-59 months on longitudinal changes in the intestinal microbiome over a 6-month period. Children under 5 years of age will be randomized to a single dose of azithromycin or placebo. *We hypothesize that children randomized to a single dose of azithromycin will exhibit a significant difference in the intestinal microbiome after a 6-month period, compared to those randomized to placebo.*

Determine the effect of a single dose of azithromycin for children aged 8 days-59 months on child growth over a 6-month period. *We hypothesize that a single dose of azithromycin will significantly increase child growth over a 6-month period, compared to single dose of placebo.*

2 Statistical Analysis

2.1 Planned Analyses

2.1.1 Longitudinal Trial

Four hundred fifty children between the ages of 8 days and 59 months may be enrolled in the trial and be offered placebo or azithromycin in a masked fashion. Relevant indicators will be collected using whole blood venipucture, nasopharyngeal swabs, fresh frozen stool, rectal swabs, and anthropometry measurements at days 0, 14, and 180. Children followed up within day 120 and day 240 will be included in primary day 180 analysis. Fifty randomly

selected children (25 per arm) will have an additional rectal swab collected at days 2, 4, 6, 8, 10, 12 post-treatment.

Primary Analyses. Pairwise comparison of *Campylobacter*, *C. upsaliensis* and *C. hominis* using Fisher's exact test, corrected for multiple comparisons using Holm-Bonferroni method.

We will compare profile of *Campylobacter*, *C. upsaliensis* and *C. hominis* based on L_1, L_2 distance, and PERMANOVA. We will compare diversity between arms at days 14 and 180 separately, using Shannon's Index and Simpson's Index.

Statistical considerations.

- Model adequacy will be checked by examination of residuals or other goodness of fit tests as needed. Inadequate model fit will prompt us to report alternative models.
- Multiple imputation will be used in case of missing baseline covariates (if applicable). Missing outcome variables will be handled by sensitivity analysis and reporting of conditional results.
- False Discovery Rate will be calculated for each estimated significant effect.

Secondary analyses.
Note that participants outside of WHO Child Growth Standards for WAZ (-6 to +5 SD), HAZ (-6 to +6 SD), or WHZ (-5 to +5 SD) will be excluded from all anthropometric analyses.

***S. pneumoniae* carriage and macrolide resistance** Separate Fisher's exact test at days 14 and 180.

lytA occurence Separate Fisher's exact test at days 14 and 180.

erm B, mef A, pp2A Separate Fisher's exact test at days 14 and 180.

N. meningitides, H. influenzae, Moraxella, S. aureus Pairwise comparison of organisms using Fisher's exact test, corrected for multiple comparisons using Holm-Bonferroni method.

PERMANOVA of L_1 and L_2 distance of bacterial reads. Shannon's and Simpson's estimated gamma diversities will be compared by arm.

Malaria Fisher's exact test of parasitemia and clinical malaria at days 14 and 180.

weight gain Defined as grams per kilogram per day (g/kg/day) will be compared between arms separately at days 14 and 180 using **linear regression**. Baseline weight is accounted for in our metric of weight gain and will not be included in the regression analysis. We will use ordinary least squares method of estimatation and permutation tests to estimate coefficient p-values.

HAZ, WAZ, WHZ, MUAC As secondary analysis, we will perform separate ANCOVA of anthropometric indicators at days 14 and 180, adjusting for baseline.

3 Sample Size Considerations

We assume a mean (standard deviation) alpha Shannon's index of 16.47 (7.12) using results from Thuy et al. [DAR+17]. Assuming 80% power and detectable effect size of 1.02 standard deviations and no loss to follow-up, we propose a sample size of 225 per group, totaling 450 study participants.

4 Randomization

The randomization will be conducted using R. The function `sample` with option `replace=FALSE` will be used to conduct the random shuffling. Note that the choice of the random number seed completely determines the randomization. To ensure the integrity of the randomization, we will use the procedure we used for MORDOR/Malawi.

Samples will be randomly grouped into 5-pools for processing, within an arm and a time point.

5 Abbreviations

ANCOVA Analysis of covariance

DSMC Data and Safety Monitoring Committee

HAZ height for age Z score

MUAC Mid upper arm circumference

SAP Statistical Analysis Plan

WAZ weight for age Z score

WHZ weight for height Z score

References

[DAR+17] T. Doan, A. M. Arzika, K. J. Ray, S. Y. Cotter, J. Kim, R. Maliki, and et al. Gut microbial diversity in antibiotic-naive children after systemic antibiotic exposure: A randomized controlled trial. *Clinical Infectious Disease*, 64, 2017.

6 Revision History

29 May 2019 Revised specification and wording of primary and secondary outcomes. Moved weight gain to secondary outcome. Clarified Bonferroni correction for certain species.

13 Jan 2020 Updated the pre-specified sample size for the sub-section of GAMIN sample (GAMIN QOD).

16 Jan 2020 Abbreviated the study name on title page.

20 Mar 2020 Added sample size considerations section and specification of day 180 time window.

08 May 2020 Specified WHO standard range for inclusion for anthropometric (child growth) analysis.

02 Jul 2020 Increased upper range of day 180 window from day 210 to day 240.

20 Oct 2021 Specified 5-pool test in sample randomization. Removed duplicated S. pneumoniae.