ACTG A5312

Statistical Analysis Plan for Pharmacology Objectives
Version 1.0

This is the ACTG A5312 Pharmacology SAP Version 1.0 with names of authors, names of publication writing team members, and analysis timeline redacted.

The Early Bactericidal Activity of High-Dose or Standard-Dose Isoniazid among Adult Participants with Isoniazid-Resistant or Drug-Sensitive Tuberculosis

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1 Introduction

1.1 Purpose

This Pharmacology Statistical Analysis Plan (SAP) describes the pharmacology-related primary and secondary outcomes measures of ACTG A5312 that will be included in the primary manuscript, and that address the pharmacology-related primary and secondary objectives of the study. The Pharmacology SAP outlines the general statistical approaches that will be used in the analyses to address these objectives. It has been developed to facilitate discussion of the statistical analysis components among the study team, and to provide agreement between the study team, pharmacometricians, and statisticians regarding the statistical analyses to be performed and presented in the pharmacology statistical analysis report. It also describes the results for the primary and secondary outcome measures that will be posted on ClinicalTrials.gov.

An original component of the EBA study, which had participants infected with TB with *inhA* mutations take one of three doses of INH (5, 10, or 15 mg/kg daily) and participants infected with DS-TB take standard-dose INH (5 mg/kg daily), was terminated early by the TB TSG on May 4, 2018. With protocol version 3.0, dated August 21, 2018, participants infected with TB with *katG* mutations are also included in the EBA part of the study (Step 2), and randomized to one of the two treatment groups (15 or 20 mg/kg daily INH). This statistical analysis plan is based on protocol version 3.0.

Regarding including participants infected with TB with *katG* mutations taking 15 or 20 mg/kg daily into Step 2 of the study (protocol version 3.0): Analysis of these data the will be conducted after 20 eligible participants in this group complete the 7-day treatment period, and data become available. Analysis will be similar to what is described here for Groups 1 (participants infected with TB with inhA mutations) and 2 (participants infected with drug-sensitive TB, with neither inhA nor *katG* mutations).

Version 1.0 of this Pharmacology Statistical Analysis plan will be used for submission of results to ClinicalTrials.gov. Results are required to be submitted within one year of the primary completion date (PCD), which is the date the last participant with *katG* mutations is examined for the purposes of data collection for the primary outcome measure.

1.2 Key Updates to the SAP

Version	Changes Made	Rationale	Effective Date
1.0	(Original version)	(Original version)	November 21, 2022

2 Protocol Overview

2.1 Study Design (Protocol Version 3.0)

This is a two-stage, two-step, phase IIa, open-label, randomized clinical trial examining the bactericidal activity (EBA) of (1) isoniazid (INH) at three different doses for treatment of isolates with *inhA* mutations; (2) INH at two different doses for treatment of isolates with *katG* mutations; and (3) INH at standard dosing

among patients with drug-sensitive TB (DS-TB). In addition, the association between the EBA among patients with DR-TB and pharmacokinetic (PK) parameters will be examined.

The study will be conducted in 2 stages. Stage 1 will be a pilot for determination of feasibility (accrual and speed) and sample size verification, and Stage 2 will be the main study. During Stage 2, participants with acid fast bacilli (AFB) smear-positive pulmonary TB with a Mycobacterium tuberculosis isolate with an *inhA* mutation, which generally confers low-level INH resistance, will be randomized to receive INH at 5, 10, or 15 mg/kg daily for 7 days (Group 1). Participants with an M. tuberculosis isolate with a *katG* mutation (with or without an *inhA* mutation), which associated with high-level resistance, will be randomized 1:1 to receive either INH 15 mg/kg or 20 mg/kg daily for 7 days (Group 3). During Stage 1, participants with M. tuberculosis harboring neither *inhA* nor *katG* INH resistance mutations (Group 2) will not receive study treatment, but a maximum of 64 in this group will have a sputum sample collected for MIC determination. During Stage 2, Group 2 participants will be enrolled as a positive control group and receive INH at 5 mg/kg daily for 7 days. Among patients receiving study treatment, serial sputum samples, including quantitative cultures and liquid cultures, will be collected daily between pre-entry and Day 7 to determine EBA of the study treatment over the treatment period.

Each stage consists of two steps. The goal of Step 1 is to determine the MIC distribution of M. tuberculosis strains among participants with DR-TB or DS-TB. The goal of Step 2 is to examine the treatment effect of INH at different doses among participants with an *inhA* mutation (in both stages), a *katG* mutation (in Stage 2 only) or DS-TB (in Stage 2 only). In Step 1, a spot sputum sample will be collected from all eligible participants for acid fast bacilli (AFB) microscopy, for genotypic determination of INH resistance (*inhA* or *katG* mutations), and for phenotypic determination of INH MIC. In Step 2, eligible participants will be administered INH daily for 7 days and have serial overnight sputum sampling for estimation of participant-specific EBA (both quantitative and liquid cultures). Intensive PK samples for INH quantification, and blood and saliva samples for NAT2 determination, will also be collected in Step 2.

Participants who are screened for the study, but are not eligible to receive study treatment will be referred without delay for appropriate treatment. Those who are registered or randomized to receive treatment will be referred without delay to appropriate treatment after completing study treatment (no later than Day 10).

SAMPLE SIZE: Among participants eligible to participate in Step 1 only, accrual targets are 70, 64, and 84 participants from Groups 1, 2, and 3, respectively. These participants may be enrolled during Stages 1 or 2.

Among participants eligible to enroll in Step 1 and Step 2, accrual targets are as follows:

- Group 1: 48 evaluable participants (16 completing treatment in each dosing cohort) from Stage 1 and Stage 2 combined. During Stage 1, 15 Group 1 participants were enrolled (5 per dosing cohort) as of March 26, 2015. Stage 2 opened and an additional 33 Group 1 participants will be randomized (11 participants added to each cohort, for a total of 16 participants per cohort)
- Group 2: 16 evaluable participants will be followed as a fourth Step 2 cohort, enrolled during Stage 2.
- Group 3: 20 evaluable participants (10 completing treatment in each dosing cohort) will be randomized.

2.2 Major Protocol Revisions

Participants enrolled under three versions of the protocol. Here we summarize the main purposes of the protocol version changes and the LOAs.

- Protocol Version 1.0 (dated October 25, 2015)
- Protocol Version 1.0 LOA#1 (dated May 4, 2013) – approved on September 17, 2015

  The main purpose was to modify the protocol to reflect the decision to conduct A5312 as a non-IND study.

- Protocol Version 1.0 LOA #2 (dated January 16, 2015) – approved on September 17, 2015

  The main purpose was to relax the Step 2 entry criteria to improve recruitment. The changes included, 1) HIV-positive candidates with CD4+ cell count of ≥50 cells/mm^3 (instead the original cut off of 200 cells/mm^3) are eligible for the study, and 2) The exclusion of current treatment, or treatment within 30 days prior to entry, with antiretroviral therapy (ART) or expected to initiate ART within 8 days after Step 2 entry has been removed.

- Protocol Version 1.0 LOA #3 (dated April 10, 2015)

  The main purpose was to to increase the target accrual for Groups 1 and 2 during Step 1.

- Protocol Version 2.0 (dated August 18, 2015) – approved on February 4, 2016

  The main changes were to increase the target accrual for Groups 1 and 2 during Step 1 (the target accrual for Groups 1 and 2 during Step 2 remains the same), to change the consent forms, to change the inclusion criteria for Group 3 to allow participants who have a *katG* mutation with or without an *inhA* mutation to enter study, and update protocol for the completion of Stage 1.

- LOA #1 after Protocol Version 2.0 (dated October 2016) – approved by the TB TSG Steering on September 28, 2016

  The main changes were to relax the exclusion criteria on usage of second-line anti-TB drugs and/or antibiotics intended for bacterial treatment prior to Step 1 screening (relaxed Section 4.4.2 and removed Section 4.4.3 of the protocol).

- Protocol Version 3.0 (dated August 21, 2018)

  The main change was to enroll participants with an *M. tuberculosis* isolate with a *katG* mutation (with or without an *inhA* mutation), associated with high-level resistance for Step 2 for the bactericidal activity analysis. During Stage 2, participants with an *M. tuberculosis* isolate with a *katG* mutation who meet Step 1 and Step 2 entry criteria are to be randomized 1:1 to receive either INH 15 mg/kg or 20 mg/kg daily for 7 days. Participants who do not meet Step 2 criteria will be enrolled in Step 1 only.

- LOA #1 after Protocol Version 3.0 (dated August 3, 2020)

This amendment was implemented to reopen the study to screening and accrual following closure due to COVID-19 on March 27, 2020 by the ACTG Network.

In addition to the LOA, five Clarification Memos (CMs) were issued after protocol version 1.0 was released, three CMs were issued after protocol version 2.0 was released, and two CMs were issued after protocol version 3.0 was released. The list below summarizes the main purpose of each CM.

- CM#1 on Protocol Version 1.0 (dated July 25, 2013)
  The main purpose was to clarify the sample size descriptions in Section 9.4 and 9.6.2.5.

- CM#2 on Protocol Version 1.0 (dated September 24, 2013)
  The main purpose was to remove language regarding non-inferiority testing from the protocol.

- CM#3 on Protocol Version 1.0 (dated November 13, 2013)
  The main purpose was to remove secondary objective 1.3.5 and the corresponding endpoint (9.2.2.5) and analysis (9.6.2.5).

- CM#4 on Protocol Version 1.0 (dated January 13, 2014)
  The main purpose was to clarify that Brooklyn Chest Hospital will participate in Stage 1 of the protocol.

- CM#5 on Protocol Version 1.0 (dated February 4, 2014)
  The main purpose was to update the biohazard containment section to include instructions on the transport of Mycobacterium tuberculosis material.

- CM#1 on Protocol Version 2.0 (dated September 14, 2015)
  The main purpose of this memo was to remove ethambutol (EMB) resistance testing from the study.

- CM #2 on Protocol Version 2.0 (dated November 17, 2015)
  The main purpose of this memo was to add PK sampling windows to the study.

- CM #3 on Protocol Version 2.0 (dated January 6, 2016)
  The purpose of this memo was to clarify that antiretroviral medications were no longer prohibited as per the eligibility criterion change made with A5312 Version 1.0, Letter of Amendment #2.

- CM #1 on Protocol Version 3.0 (dated December 12, 2019)
  The main reason for this memo was to clarify the target accrual for the study.

- CM #2 for protocol Version 3.0 (dated February 25, 2020)
  The reason for this memo was to clarify the washout period for drugs with anti-TB activity.

2.3 Primary Pharmacology Objective

2.3.1 Determine the association between the area under the curve (AUC)/MIC of INH and the EBA

of INH among participants with smear-positive pulmonary TB.

2.4 Secondary Pharmacology Objectives

2.4.1 Determine the steady state pharmacokinetics (PK) of INH among participants with sputum smear-positive pulmonary TB taking 5, 10, 15, or 20 mg/kg daily, taking into account INH acetylator status (N-acetyltransferase 2 [NAT2] genotype).

2.4.2 Determine and describe the distribution of MICs of M. tuberculosis isolates with genotypic evidence of low-level INH resistance (*inhA* mutations), high-level INH resistance (*katG* mutations), or neither of these mutations among participants with smear-positive pulmonary TB.

2.4.3 Estimate the proportion of participants infected with a TB strain that has an *inhA* or *katG* mutation that will achieve a target AUC/MIC that is associated with clinically relevant reductions in mycobacterial burden defined as at least 50% the EBA0-7 of standard-dose INH when given to participants with DS-TB, by dose.

2.5 Exploratory Pharmacology Objectives

2.5.1 Evaluate the ability of a new PCR method that uses salivary samples to correctly characterize NAT2 genotype compared to standard NAT2 genotyping methods using blood samples and sequencing methodology.

This objective will be addressed in a separate SAP.

3 Definitions

3.1 Endpoint Definitions

Intensive PK sampling was conducted on study day 6, when the participant will have reached steady-state. Sample times were pre-dose, and 0.5, 1, 2, 4, 6, 8, 12 and 24 hours after the dose taken in the clinic. Concentrations from intensive sampling are used to estimate participant specific steady-state INH PK parameters, using noncompartmental analysis. Details are as follows:

- Minimum concentration, C_{min}: the minimum plasma concentration observed in the 24-hour dosing interval.
- Maximum concentration, C_{max}: the maximum plasma concentration observed in the 24-hour dosing interval.
- Time of maximum concentration, T_{max}: the time at which C_{max} occurred. If there is a tie for maximum concentration, T_{max} is assigned the earliest such sample time.
- Area under the concentration-time curve from 0-24 hours, $AUC_{0\text{-}24}$: the AUC will be calculated using the linear trapezoidal rule (the sum of adjacent trapezoids in the concentration-time curve). In calculation of AUC, the estimated concentration at precisely 24 hours ($\hat{C}_{24}$) will be used, where $\hat{C}_{24}$ is estimated using the observed concentrations and times for the samples scheduled to be collected at 12 and 24 hours after the in-clinic dose. A simple linear regression model is fit to these two points. The concentration at exactly 24 hours is estimated from the intercept and slope estimates. (This approach guards against a upward/downward bias in AUC when the final sample was collected before/after 24 hours. In the case where the sample time was exactly 24 hours, the observed and estimated concentrations at 24 hours are identical.)
- Elimination half-life, $T_{½}$: assuming exponential decay, participant-specific half-life will be estimated by first fitting a log-linear regression (log base e) to the 3 or more observed concentrations (in the

participant's concentration-time data) that occur after (ie, not including) Cmax, provided all such concentrations are monotonically decreasing; if 3 such terminal concentrations are not available, half-life will not be calculated. The value of half-life is given by $\ln(2)/k_e$, where k_e, is the participant-specific estimated slope with the sign reversed (ie, expressed as a positive number). The values below limit of quantification (BLQ) will be removed when calculating half-life.

- Apparent oral clearance, CL/F: estimated as the (daily) dose amount divided by the AUC (after converting numerator and denominator to like units).

Activity parameters **EBA0-7(CFU)** and **EBA0-7(TTD)** are defined in the Primary Statistical Analysis Plan, version 1.0.

3.2 Analysis Populations

Pharmacology population: The pharmacology population is defined as those participants: (1) whose intensive PK sampling began on study day 6, (2) who were fasted (nothing by mouth except water and study medications from 2 hours pre-dose to 1 hour post-dose) at the time of the pre-dose sample, (3) who had taken at least 2 INH doses within the 52-hour period prior to the pre-dose sample, and (4) for whom concentrations are available for both the 1- and 2-hour sample (so as not to miss T_{max}).

Efficacy population: The efficacy population is limited to participants in the pharmacology population for whom INH AUC or INH MIC is available, and for whom EBA0-7(CFU) and/or EBA0-7(TTD) are available.

4 Statistical Methods

4.1 General Considerations

For visit schedule and analysis windows, see the "A5312_final_analysis_SAP_v1.0.docx."

Categorical data will be summarized using N (%). PK parameters and MIC will be summarized using N, min, Q1, median, Q3, max. Other continuous data will be summarized using N, min, Q1, median, Q3, max, mean and standard deviation (SD).

4.2 Analyses to address the primary pharmacology objective

Determine the association between the area under the curve AUC of INH and the EBA of INH among participants with smear-positive pulmonary TB (post hoc change from AUC/MIC to AUC[1]).

Pharmacometricians will address this objective as follows:

The drug-induced bacterial killing for sputum solid culture colony-forming unit (CFU) and growth of bacteria in Mycobacteria Growth Indicator Tube (MGIT) for time to positivity (TTP) will be jointly modelled using nonlinear mixed-effects modelling. A mono-exponential model will be used to describe the bacterial load decline in CFU, while an exponential growth model will be used to describe the TTP in MGIT. The two models will be joined by using the predicted CFU bacterial load in the sputum to initialize the MGIT inoculum. An Emax model will be used to relate isoniazid AUC to the kill rate of the bacteria in the CFU. If the unavailable CFU data in Group 3 participants results in the underperformance of the above-described

[1] Regarding the post hoc change from AUC/MIC to AUC as the explanatory variable, in the time since the protocol was written, the team has reconsidered the interpretability of MIC testing using the 1% agar method (which was used in protocol versions 1.0 and 2.0), and is reporting only MICs obtained using Thermo Fisher Sensitre MYCOTB plates (which was used in protocol version 3.0). Only participants with a *katG* mutation were treated with INH and had MIC testing using this method. In addition, the low data availability for MICs limits its usefulness in modeling.

model, a linear mixed-effects model will be used to evaluate the entire TTP profile, exploring the effect of isoniazid area under concentration curve (AUC_{0-24}) covariation with longitudinal changes in TTP.

For clinicaltrials.gov, the outcomes measures (INH AUC, EBA_{0-7}(CFU) and EBA_{0-7}(TTP)) will be summarized.

4.3 Analyses to address secondary pharmacology objective 1

Determine the steady state pharmacokinetics (PK) of INH among participants with sputum smear-positive pulmonary TB taking 5, 10, 15, or 20 mg/kg daily, taking into account INH acetylator status (N-acetyltransferase 2 [NAT2] genotype).

Pharmacologists will address this objective for the submission of study results to clinicaltrials.gov as follows:

Participant-specific PK parameters will be estimated using noncompartmental methods.

For the PK parameters C_{min}, C_{max}, and AUC_{0-24h}: N, min, max, median, and the 25% and 75% percentiles will be tabulated for the following categories listed below. Additional parameters (T_{max}, CL/F, $T_{½}$) and statistics (min, Q1, median, Q3, max) will also be summarized but are not required for clinicaltrials.gov submission.

Resistance/dose groups

1. Group 1: *inhA* resistance mutation
    a) 5 mg/kg/day
    b) 10 mg/kg/day
    c) 15 mg/kg/day
2. Group 2: neither *inhA* nor *katG* resistance mutation
    a) 5 mg/kg/day
3. Group 3: *katG* resistance mutation
    a) 15 mg/kg/day
    b) 20 mg/kg/day

Additional analysis stratified by NAT2 acetylator status will be done, but will not be included in clinicialtrials.gov.

4.4 Analyses to address secondary pharmacology objective 2

Determine and describe the distribution of MICs of M. tuberculosis isolates with genotypic evidence of low-level INH resistance (inhA mutations), high-level INH resistance (katG mutations), or neither of these mutations among participants with smear-positive pulmonary TB.

Statisticians will address this objective for the submission of study results to clinicaltrials.gov as follows:

Descriptive statistics of MICs with N missing, N, median (IQR), min, and max will be summarized by mutation group (*inhA* mutation, *katG* mutation, or neither of these mutations).

Analyses to address secondary pharmacology objective 3

Estimate the proportion of participants infected with a TB strain that has an inhA or katG mutation that will achieve a target AUC/MIC that is associated with clinically relevant reductions in mycobacterial

burden defined as at least 50% the EBA0-7 of standard-dose INH when given to participants with DS-TB, by dose.

Post hoc change: Given the complexity of the EBA PK-PD model and the associated variability, technical issues with MIC determination using the 1% agar solution method used for participants with inhA mutated or DS-TB (Groups 1 and 2) and non-comparability of those results to MIC determination using Thermo Fisher Sensititre MYCOTB plates (used for Group 3), the analysis team decided that establishing a target based on AUC or AUC/MIC would be problematic. Instead, the median drop in log10 CFU in participants with DS-TB (Group 2) on day 7 of treatment was identified and divided by two, to arrive at a target EBA. Analysis will focus on estimating the proportions of participants in each group/dose who achieve that target.

Pharmacometricians will address this objective for the submission of study results to clinicaltrials.gov as follows:

The DS-TB arm day 7 drop per mL in log10 CFU was 1.3 log10 CFU; half of this drop is 0.65 log10 CFU which will be the target change in EBA from day 0 to day 7. Analysis will focus on reporting the proportion of participants in each group/dose whose estimated EBA was 0.65 log10 CFU/mL or larger (median of bootstrap simulations) along with a bootstrap 95% confidence interval (5^{th} and 95^{th} percentiles of bootstrap simulations). In the simulations, a distribution of NAT2 genotypes will be assumed which matches the distribution observed in Group 1 and 2 participants (14% rapid, 58% intermediate, and 28% slow acetylators[2]).

[2] Gausi K, Ignatius EH, Sun X, Kim S, Moran L, Wiesner L, et al. A Semimechanistic Model of the Bactericidal Activity of High-Dose Isoniazid against Multidrug-Resistant Tuberculosis: Results from a Randomized Clinical Trial. Am J Respir Crit Care Med [Internet]. 2021 Dec 1 [cited 2021 Sep 15];204(11):1327–35. Available from: www.atsjournals.org.