This is the Statistical Analysis Plan Version 2.0 for A5366 with names of authors, names of publication writing team members and analysis timeline redacted.

A5366

Primary Statistical Analysis Plan

Version 2.0

Selective Estrogen Receptor Modulators to Enhance the Efficacy of Viral Reactivation with Histone Deacetylase Inhibitors

ClinicalTrials.gov Identifier: NCT03382834

(Based on A5366 protocol version 1.0, CM #1 - #5 and LOA #1)

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Table of Contents

TABLE OF CONTENTS **2**

1 INTRODUCTION **4**
1.1 Purpose **4**
1.2 Key Updates **4**

2 PROTOCOL OVERVIEW **5**
2.1 Study design **5**
2.2 Hypothesis **5**
2.3 Study Objectives **5**
2.4 Outcome Measures **6**

3 DEFINITIONS **6**
3.1 Time on Study **6**
3.2 Analysis Populations **6**

4 STATISTICAL METHODS **7**
4.1 Primary Analyses **7**
4.2 Secondary Analyses **7**

5 REPORT COMPONENTS **9**
5.1 Screening (if data available in SDTM) **9**
5.2 Accrual **9**
5.3 Eligibility Violations (if data available in SDTM) **9**
5.4 Baseline Characteristics **9**
5.5 Study Status **10**
5.6 Treatment Status **10**
5.7 ART Status **10**
5.8 Day 28 HIV-1 RNA level **10**
5.9 Primary Analyses **11**
5.9.1 Safety: Adverse Events (AEs) 11
5.9.2 Efficacy: Cell associated RNA 11
5.10 Secondary Analyses **11**
5.10.1 Cell associated HIV DNA 11
5.10.2 SCA 11
5.11 Exploratory Analyses **11**
5.11.1 Integrated HIV-1 DNA levels, HIV p24 antigen expression, and ex vivo HIV-1 RNA expression (EDITS) 11
5.11.2 HIV-specific immunity and activation: HIV-1-specific immune responses, T-cell activation, T-cell exhaustion markers 11
5.11.3 Histone H3 acetylation 11
5.11.4 Pharmacokinetic parameters of vorinostat and tamoxifen 11
5.11.5 Measures of innate cellular markers and soluble factors 12

5.11.6 Hormone levels (estradiol, estrone, progesterone) 12

6 CORE MANUSCRIPT WRITING TEAM 13

7 TIMETABLE FOR PRIMARY ANALYSIS AND MANUSCRIPT PREPARATION 13

1 Introduction

1.1 Purpose

This Primary Statistical Analysis Plan (SAP) describes the primary and secondary outcome measures of A5366 that will be included in the primary manuscript addressing the major primary and secondary objectives of the study, as well as the primary and secondary outcome measures for which results will be posted on ClinicalTrials.gov. The Primary SAP also outlines the general statistical approaches that will be used in the analysis of the study. It has been developed to facilitate discussion of the statistical analysis components amongst the study team; and to provide agreement between the study team and statisticians regarding the statistical analyses to be performed and presented in the primary statistical analysis report.

1.2 Key Updates

Version 1.0 of the A5366 Primary Statistical Analysis Plan is based on A5366 protocol version 1.0, CM #1, CM #2 and LOA #1.

Version 2.0 of the A5366 Primary Statistical Analysis Plan is based on A5366 protocol version 1.0, CM #1, CM #2, CM #3, CM #4, CM #5 and LOA #1, and team discussions:

- Updated efficacy population definition
- Clarified analyses for secondary and exploratory outcomes
- Updated to focus on Step 1 analyses

2 Protocol Overview

2.1 Study design

A5366 is a randomized, open-label, exploratory study of post-menopausal women with HIV and virologic suppression on antiretroviral therapy (ART) assessing the effects of tamoxifen exposure in combination with vorinostat compared to vorinostat treatment alone on viral reactivation.

The study population is post-menopausal women (age 18-65) with HIV and virologic suppression on ART. The planned sample size is 30 participants, who will be randomized (2:1) to Arm A or Arm B to receive the following regimens:

Arm A:
Days 0-38: Tamoxifen 20 mg orally once daily
Day 35: Vorinostat 400 mg orally once daily
Day 38: Vorinostat 400 mg orally once daily

Arm B:
Days 0-38: Observation period (no tamoxifen)
Day 35: Vorinostat 400 mg orally once daily
Day 38: Vorinostat 400 mg orally once daily

A5366 has 2 Steps: participants will be followed for 65 days in Step 1, and an additional 240 weeks in Step 2 (long-term safety follow-up). The study primary efficacy outcome, however, is based on data collected during Step 1.

2.2 Hypothesis

Treatment with the selective estrogen receptor modulator (SERM) tamoxifen will enhance the ability of the histone deacetylase inhibitor (HDACi) vorinostat to reverse HIV-1 latency. In participants with HIV on ART, combination therapy with tamoxifen and vorinostat will lead to higher levels of HIV-1 cell-associated RNA than will vorinostat alone.

2.3 Study Objectives

The primary objectives of A5366 are the following:

- Determine the safety and tolerability of combined therapy with the SERM tamoxifen and the HDACi vorinostat among HIV-infected post-menopausal women.
- Determine the level of HIV-1 reactivation following treatment with vorinostat versus tamoxifen and vorinostat as measured by cell associated HIV-1 RNA (CA-RNA) in CD4+ T cells (primary efficacy endpoint).

The secondary objectives of A5366 are the following:

- Evaluate the impact of therapy with vorinostat versus a combination of tamoxifen and vorinostat on residual viremia, as measured by a single copy assay.
- Assess the impact of therapy with vorinostat versus a combination of tamoxifen and vorinostat on markers of HIV-1 persistence, as measured by total DNA levels.

2.4 Outcome Measures

The primary outcome measures are: (protocol section 10.2.1)

- Safety: Occurrence of a new Grade ≥3 adverse event that is considered definitely, probably, or possibly related to study treatment (as judged by the core protocol team)
- Efficacy: Change in cell-associated HIV-1 RNA in CD4+ T cells from baseline to the day 38 post-vorinostat time point

The secondary outcome measures are: (see protocol section 10.2.2):

- Single copy plasma HIV-1 RNA assay
- Total HIV-1 DNA levels

3 Definitions

3.1 Time on Study

“Day 0” is defined as the day of study randomization

“Baseline” refers to parameters measured on or before randomization/on-study date.

Days and weeks on Step 1 will be based on Step 1 registration day through “day 0”. Days and weeks on Step 2 will be based on Step 2 registration through end of study.

3.2 Analysis Populations

“Study treatment” is defined as vorinostat and tamoxifen.

“Safety population” is defined as the subset of participants who have been exposed to the study treatment.

“Efficacy population” was originally defined as the subset of participants who have received the full study treatment, and do not have interruption of ART or confirmed HIV-1 RNA ≥ 200 copies/mL between the pre-treatment and post-treatment (Day 38) time points. Based on plasma HIV-1 RNA testing by SCA, one participant had a pre-entry HIV-1 RNA level = 28,452 copies/mL and entry HIV-1 RNA level = 164 copies/mL (11,000 and 33 copies/mL, respectively, by Roche cobas 6800/8800); after team discussion it was decided that this participant would be excluded

from all efficacy analyses. In addition, a site erred and did not collect PBMCs/plasma at Day 38 Hour 5 (post vorinostat) for one participant; because this is the timing of the primary efficacy outcome measure, after team discussion it was decided that this participant would also be excluded from all efficacy analyses.

4 Statistical Methods

4.1 Primary Analyses

For the primary safety analysis, AEs attributed to study treatment based on core protocol team review will be summarized separately for the two treatment arms. All participants who have been exposed to the study treatment will be included in this analysis. The primary safety outcome (see protocol section 10.2.1.1) will be summarized separately by treatment arm, including a 1-sided 95% confidence interval using exact binomial methods. Tolerability will be assessed by summaries of early treatment discontinuations, including reasons, separately by treatment arm.

Because the aim of this pilot study is to assess biologic activity, the primary efficacy analysis will be as-treated, limited to participants who received the full study treatment; see efficacy population above. The primary efficacy outcome (see protocol section 10.2.1.2) is the change from baseline (average of the pre-entry and entry time points) to the post-vorinostat time point (5 hours following the second vorinostat dosing) of CD4+ T cell-associated HIV-1 RNA. The change (and the average of the pre-treatment measurements) will be done on log10-transformed measurements; results below assay limits will be set to half the lower limit prior to statistical analysis. (Only a minority of results, estimated to be 10%, are anticipated to be below assay limit; Dr. Athe Tsibris, Brigham and Women's Hospital, personal communication.) The primary analysis to assess whether tamoxifen enhances the effect of vorinostat will be based on a 2-group t-test. The level of the test for this pilot study will be at a 1-sided alpha=0.1.

4.2 Secondary Analyses

A supplemental analysis of the primary efficacy outcome will also be performed, in which results below assay limit will be addressed using censored-data longitudinal analysis methods (see Riddler at al, JID 2016).

Secondary analyses of HIV-1 persistence measures, immunologic measurements and histone acetylation will be analyzed similarly to the primary efficacy outcome (see protocol section 10.6.1) but using 2-sided alpha of 0.05 for each comparison. It is anticipated that measures of HIV-1 persistence will be log-transformed prior to analysis. Plots (including participant-specific longitudinal profiles) and descriptive data summaries will also be presented, by treatment arm, including 95% confidence intervals of changes from baseline to post-baseline time points. This will address, for example, the potential effects of tamoxifen alone on HIV-1 reservoir measures. Because a substantial fraction of single copy HIV-1 RNA measurements are below assay limit, changes will be evaluated using statistical methods for longitudinal censored data (Vaida F, Liu L. Fast implementation for normal mixed effects models with censored response. J Comput Graph

Stat 2009; 18:797-817). The proportion below assay limit, by arm, will also be summarized longitudinally.

Correlations between measurements will also be examined, in particular correlations between changes in the cell-associated RNA and changes in the other virologic and immunologic measures.

While measurements at pre-entry and entry will be averaged to assess treatment effects (see protocol section 10.6.1), supplemental descriptive analyses will summarize the biologic and measurement variability, and stability, of the virologic and immunologic measures while on stable suppressive ART (i.e., prior to treatment intervention).

5 Report Components

Analysis summaries will be presented for each arm and overall.

For the final analysis, lists will be indexed by the alternate patient identifier (**SUBJID** variable), and visit week/date as needed. For interim analyses, the database patient identifier (**USUBJID** variable) will be used to facilitate cross-referencing with site communications.

NOTE: Each table/list/figure in the reports will be annotated with the name and location of the program that created it, and the creation date.

5.1 Screening (if data available in SDTM)

Table(s) summarizing:

- Number (%) screened by month/year
- Number (%) screened by site
- Number of screen failure reasons

5.2 Accrual

Table(s) summarizing:

- Number (%) of participants enrolled by month/year
- Number (%) of participants enrolled by site

5.3 Eligibility Violations (if data available in SDTM)

- Table summarizing violations of eligibility criteria by site (if any).
- List summarizing details of exclusions from analyses (if any).

5.4 Baseline Characteristics

Table summarizing:

- Sex
- Age at entry date
- Self-reported race/ethnicity
- IV drug use
- Entry ART regimen
- Years since first ARV use
- Entry HIV-1 RNA level
- Screening CD4 cell count
- Nadir CD4 cell count

For categorical variables: showing number (%) by category.

For continuous variables: showing median, 25th and 75th percentiles, min, and max.
Age will be summarized both as categorical (<50, 50-59, ≥60) and continuous.

5.5 Study Status

Tables summarizing (Step 1):

- Number of participants enrolled to the study
- Number of participants on study
- Number of participants off study
    - Reasons off study

5.6 Treatment Status

- Tamoxifen status (for arm A participants only)
    - Number of participants on arm A without a tamoxifen treatment record (did not initiate)
    - Number of participants who completed tamoxifen per protocol
    - Number of participants who discontinued tamoxifen early
        - Reasons for early tamoxifen discontinuation

- Vorinostat status (for all participants)
    - Number of participants who received 2 doses of vorinostat
    - Number of participants who received <2 doses of vorinostat
        - Reasons for receiving <2 doses of vorinostat

5.7 ART Status

Table(s) summarizing:

- ART discontinuations (if any)
- ART interruptions (if any)
- ART modifications (if any)

5.8 Day 28 HIV-1 RNA level

Table(s) summarizing:

- Number of participants with plasma HIV-1 RNA level ≥ 200 copies/mL at day 28
    - Number of participants who returned for a repeat HIV-1 RNA level
        - Summary of the repeat HIV-1 RNA level
    - Number of participants who have not (yet) returned for a repeat HIV-1 RNA level
        - Distribution of elapsed time from first HIV-1 RNA level ≥ 200 copies/mL result until data retrieval date

5.9 Primary Analyses

5.9.1 Safety: Adverse Events (AEs)

List of all AEs: participant ID, randomized Arm, age, days from study entry to onset day, AE description, AE grade, AE outcome, and safety core team attribution to study treatment.

5.9.2 Efficacy: Cell associated RNA

Table of CA-RNA levels at each time point (baseline, Day 28, Day 35, Day 38, Day 45, Day 65): median, 25th and 75th percentiles, min, max, N.

Table of change from Baseline to Day 38: median, 25th and 75th percentiles, min, max, mean, N. 95% confidence interval separately by arm. P-value from 2 group t-test and point estimate (mean) and 95% confidence interval for the difference between arms in change from baseline to Day 38.

Figure of CA-RNA levels over time: median, mean, 25th and 75th percentiles.

5.10 Secondary Analyses

5.10.1 Cell associated HIV DNA

5.10.2 SCA

5.11 Exploratory Analyses

5.11.1 Integrated HIV-1 DNA levels, HIV p24 antigen expression, and ex vivo HIV-1 RNA expression (EDITS)

5.11.2 HIV-specific immunity and activation: HIV-1-specific immune responses, T-cell activation, T-cell exhaustion markers

5.11.3 Histone H3 acetylation

5.11.4 Pharmacokinetic parameters of vorinostat and tamoxifen

Summaries of vorinostat levels at Day 38 (pre-dose and post-dose hour 5) will be presented overall and by Arm. The post-dose level will be compared between arms (t-test, 95% confidence interval on difference between Arms). Summaries of tamoxifen levels by week will be presented for Arm A only. The average of tamoxifen levels at Day 35 and Day 38 will be summarized and associated with study outcomes. Initial analyses will correlate vorinostat levels (Day 38 post-dose hour 5) with change in histone H3 antibody levels pre-vorinostat to post-vorinostat (Day 28 to Day 38 post-dose hour 5). Additional pharmacokinetic parameters will also be summarized and associated with study outcomes. The specifics of these

pharmacodynamics analyses will be developed by the study team after review of the pharmacokinetic data and the results of the primary and secondary study analyses.

5.11.5 Measures of innate cellular markers and soluble factors

5.11.6 Hormone levels (estradiol, estrone, progesterone)

Due to skewed distributions of hormone levels, initial summaries will be on log-transformed hormone levels. Further analyses of hormone levels may be performed upon team review of these summaries.

6 Core Manuscript Writing Team

Protocol Co-chairs, Statisticians, Clinical Representatives and selected Investigators.

7 Timetable for Primary Analysis and Manuscript Preparation

Note that the actual calendar dates will only be filled in in the final version of the SAP

Information redacted