A5369

Primary Statistical Analysis Plan

HIV-1-Gag Conserved-Element DNA Vaccine (p24CE) as Therapeutic Vaccination in HIV-Infected Persons with Viral Suppression on Antiretroviral Therapy

ClinicalTrials.gov Identifier: NCT03560258

Version 2.0

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1 Introduction

This Primary Statistical Analysis Plan (SAP) describes the primary and secondary outcome measures and general analytic approaches of ACTG A5369 that will be included in the primary manuscript or submitted to ClinicalTrials.gov. 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 (SAR). Detailed outlines of tables, figures, and coding descriptions that will be included in the Primary SAR are included in the Analysis Implementation Plan (AIP). A separate SAP for Other Objectives will be developed when results for the Primary and Secondary Objectives are known, and analyses will be presented in a separate report. Data for the Primary SAR will be downloaded once the last participant has completed 48 weeks of follow-up, all queries have been resolved, and the database frozen for analysis.The Primary SAR will be used for submission of results to ClinicalTrials.gov. Results for primary outcomes are required to be submitted within one year of the primary completion date (PCD), which is the date that the final participant is examined for the purposes of final collection of data for all of the primary outcomes. The primary immunogenicity outcome is measured at 26 weeks of participant follow-up, and the primary safety outcome data collection is completed at 48 weeks of follow-up. Thus, the PCD of this study is based on 48 weeks of follow-up. Results for secondary outcome measures for this study are due at the same time.

1.1 Summary of Key SAP Updates

Version	Changes Made	Effective Date
1.0	Original Version	8/23/2018
1.0	Protocol Amendment Review (V1.0, LOA #1) Huichao Chen	3/28/19
1.0	Protocol Amendment Review (V1.0, LOA#2) Huichao Chen	4/16/19
2.0	Per CM #2, due to the COVID-19 situation, the visit window is broadened from ±14 days to -14 days to +60 days, starting from 03/16/20. Per LOA#3, the visit window is further broadened from ±14 days to -14 days to +180 days.	09/10/20

2 Study Overview

2.1 Study Design

A5369 is a phase I/IIa, randomized, double-blind, placebo-controlled study that will evaluate the safety, immunogenicity, and preliminary assessment of efficacy of a novel vaccine encoding "conserved elements" (CE) of the HIV-1 Gag core protein, p24Gag, as a therapeutic vaccine in HIV-1 infected persons on antiretroviral therapy (ART) with the aim to induce potent virus-specific cytotoxic T lymphocytes (CTL) responses. The primary efficacy outcome will be based on HIV-specific immunologic assays at baseline and week 26.

Duration
Participants will be on study for a total of 48 weeks.

Sample Size The target sample size of 40 (20 p24CE; 10 full length Gag; 10 placebo) for the randomized arms is selected to detect a 0.54 difference in the probability that a participant has increased CE responses (0.64 for p24CE vaccine and 0.10 for placebo) between arms based on a two-sided Fisher's exact test with 80% power, 5% alpha level, and 10% inflation of the estimated sample size for loss-to-follow-up or unusable laboratory specimens. The study also has approximately 80% power to detect a difference of 0.85 versus 0.30 when comparing the p24CE and full-length Gag vaccine arms.

Population
HIV-infected individuals receiving ART with plasma HIV-1 RNA <50 copies/mL for at least 2 years (one "blip" allowed), current CD4 T cell counts >500 cells/mm3, and nadir CD4 T cell counts >350 cells/mm^3 (by documentation or candidate recall).

Randomization and Stratification
Participants will be randomized 2:1:1 to the p24CE/full-length Gag DNA vaccine arm versus full-length Gag DNA vaccine arm versus placebo arm using the permuted block method. Randomization will be stratified by indication of willingness to have the leukapheresis procedure at study screening. The active vaccines/placebo will be administered by intramuscular injection/electroporation.

Regimen

Arm 1: p24CE/full-length Gag DNA Vaccine Arm
p24CE1/2 pDNA 4 mg administered by injection/electroporation at week 0 and week 4. Then p24CE1/2 pDNA 2 mg admixed with full-length p55gag pDNA 2 mg administered by one injection/electroporation at week 12 and week 24.

Arm 2: Full-length Gag DNA Vaccine Arm
Full-length p55gag pDNA 4 mg administered by one injection/electroporation at week 0, week 4, week 12 and week 24

Arm 3: Placebo Arm
Placebo (Sodium Chloride for Injection, USP 0.9%) 1 ml administered by one injection/electroporation at week 0, week 4, week 12 and week 24.

Analysis will be performed in a superiority framework, comparing between arms the change in the number of CEs with a CD4 and/or CD8 T cell response from baseline to week 26.

Major change of the study follow-up

Due to the feasibility challenges posed by the COVID-19 situation, Clarification Memo #2 was issued to broaden the visit window from ±14 days to -14 days to +60 days, starting from 03/16/20. On April 8 2020, after further review, Letter of Amendment #3 was then issued to further broaden the visit window from +60 days to +180 days but sites were still encouraged to schedule the applicable visits within +90 days.

2.2 Hypothesis

We hypothesize that the administration of p24CE Gag DNA vaccine followed by p24CE DNA + full-length Gag DNA vaccine boost to HIV-infected persons on antiretroviral therapy (ART) will focus newly induced immune responses to immunogenic conserved epitopes of Gag and away from decoy variable epitopes, compared to the administration of full-length Gag DNA vaccine alone, and compared to placebo. We will also measure safety and effects on the latent cell reservoir of HIV-1 in the study. The vaccine regimes will be safe and well tolerated.

2.3 Study Objectives

This Primary SAP addresses the following primary and secondary objectives listed in the study protocol. The remaining study objectives in the protocol will be addressed in subsequent analysis plans.

2.3.1 Primary Objective

1. To compare the additional number of p24CEs recognized by a CD4 and/or CD8 T cell response in participants at each study arm at week 26 compared to baseline
2. To evaluate the safety of the p24CE/full-length Gag DNA vaccine and the full-length Gag DNA vaccine in HIV-infected persons receiving ART.

2.3.2 Secondary Objectives

1. To compare the percent of individual study participants in each study arm with a CD4 and/or CD8 T cell response to an increased number of conserved elements (CEs) at week 26 compared to baseline.
2. To compare the percent of individual study participants in each study arm with a CD4 T cell response to an increased number of CEs at week 26 compared to baseline.
3. To compare the percent of individual study participants in each study arm with a CD8 T cell response to an increased number of CEs at week 26 compared to baseline.
4. To compare the change in total magnitude of CD4 T cell responses against each CEs added together across study arms.
5. To compare the change in total magnitude of CD8 T cell responses against each CEs added together across study arms.

2.4 Interim Monitoring

The study will undergo interim review at least annually by an independent ACTG-appointed Study Monitoring Committee (SMC). The SMC will review accrual, baseline characteristics, conduct of the study (including premature treatment and study discontinuations), data completeness, and AEs by treatment arm. The first interim review will occur approximately 6 months after the enrollment of the first study participant, or after 20 participants enroll, whichever occurs first. An

interim review may also be convened if a concern is identified by the DAIDS clinical representative, the study chairs, or study statisticians in consultation with the team.

NOTE: The pre-specified study suspension rule is

If at any time during the study:

- Two or more participants experience a primary safety outcome that is possibly or probably related to study treatment (as judged by the core team, blinded to treatment arm), or
- One or more participants experience a primary safety outcome that is definitely related to study treatment or that is Grade 4 or death and possibly or probably related to study treatment (as judged by the Core Team, blinded to treatment arm), or
- Two or more participants experience a viral load rebound plasma HIV-1 RNA >1,000 copies/ml confirmed by a second consecutive reading,

then enrollment into the study will be temporarily suspended and the Study Monitoring Committee (SMC, unblinded to treatment assignment) will be asked to review all safety data; review the relation to study treatment of the event(s) thought by the blinded Core Team to be a primary safety outcome; and recommend how the study should proceed with respect to resuming enrollment and continuing study treatment.

Pending full SMC review, the protocol team will decide, in consultation with the SMC chair or designee, whether to continue study treatment for subjects already enrolled.

3 Outcome Measures

Per protocol Section 10.2.1, the primary outcome measures are as follows

3.1 Primary Outcome Measures

3.1.1 Immunogenicity

The number of CEs with a CD4 and/or CD8 T cell response at week 26 compared to baseline in an individual study participant. A CE response (for a participant at a given time point) is defined by a criterion (Horton et al., J Immunol Methods. 2007) that uses Fisher's exact test to compare the proportion of peptide-stimulated CD4 and/or CD8 T cells that are cytokine positive to the proportion that are cytokine positive in the unstimulated sample (negative control), and applying a stringent p-value of 10e-5 as a cutoff.

NOTE: The number of CEs with a CD4 and/or CD8 T cell response will be provided by Dr. De Rosa's lab.
NOTE: For this primary outcome, we will sum the CD4 and CD8 responses.

3.1.2 Safety

Occurrence of at least one ≥Grade 3 adverse event (AE, see definition of AE in protocol Section 7.1) except injection site pain or tenderness of less than 48 hours duration, that is possibly,

probably, or definitely related to study treatment (as judged by the core team, blinded to treatment arm) any time from the first day of study treatment until 28 days after the last study vaccine administration.

Grade 4 AEs and deaths at any time on study will be considered as a primary safety outcome.

3.2 Secondary Outcome Measures

Per protocol Section 10.2.1, the secondary outcome measures are as follows:

1. The number of CEs with a CD4 T cell response at week 26 compared to baseline in an individual study participant.
2. The number of CEs with a CD8 T cell response at week 26 compared to baseline in an individual study participant.
3. The total magnitude of CD4 T cell responses against each CE added together in each study arm at week 26 compared to baseline.
4. The total magnitude of CD8 T cell responses against each CE added together in each study arm at week 26 compared to baseline.

4 Statistical Principles

4.1 Analysis Populations

Modified Intent-To-Treat (mITT): All randomized participants who have been exposed to study treatment (i.e., at least one dose of study drug).

Per-Protocol (PP): All eligible, randomized participants who 1) received all four study vaccination / placebo administrations, and 2) did not take any medication prohibited by the study protocol on or prior to their week 26 visit (as defined per protocol section 10.7.1).

4.2 Participant Exclusion

Participants found to be ineligible for A5369 by the site, study statistician(s), or data manager will be reviewed by the study chair and vice chair for confirmation of their ineligibility.

4.3 Visit Schedule and Analysis Windows

The schedule of evaluations is given in Section 6.1 the protocol. See timing of evaluations below:

- **Screening**: evaluations must be completed within 60 days prior to randomization (i.e, entry, week 0) unless otherwise specified.
- **Pre-Entry**: evaluations must be completed at least 24 hours after screening evaluations have been completed and at least 24 hours prior to entry evaluations unless otherwise specified.
- **Entry (week 0)**: evaluations must occur at least 24 hours after pre-entry evaluations unless otherwise specified. Study entry or baseline is defined as the randomization date. The value used for baseline summaries will be the last evaluation on or before the randomization date. Participants must begin treatment within 72 hours after study enrollment.
- **Post-Entry**: evaluations occur after the study entry visit and vaccination #1 administration at week 0. The visit window for the post-entry evaluations is ± 14 days.

- **Week 4** (vaccine #2 administration)**:** weeks (2, 6]
- **Week 12** (vaccine #3 administration)**:** weeks (10, 14]
- **Week 24** (vaccine #4 administration)**:** weeks [22, 26]
- **Week 48**: evaluations will be completed at the participant's final on study visit and scheduled within a ± 14 days; weeks (46, 50].

Due to the delayed visits during the COVID-19 pandemic, starting 03/16/20, the visit window for week 24 and 48 was broadened from ±14 days to -14 days to +60 days. The broadened visit window was applicable to visits scheduled to occur before 04/30/20. Upon further review, the visit window was then broadened from ±14 days to -14 days to +180 days for visits that were planned to occur before 07/15/20. While +180 days was allowed, sites were still encouraged to schedule the applicable visits within +90 days. Thus, the new visit window for week 24 is weeks [22, 50] and week 48 is weeks [46, 74]

According to CM # 2, the week 24 and week 26 visits should remain separate and coordinated to be as close to 2 weeks apart as feasible, but can be up to 60 days apart. Thus, if the week 26 visit has to be delayed, it is better to postpone the week 24 visit until the week 26 visit can occur 2 weeks later, or as close to that interval as possible.

- Any deferred study vaccinations #2, #3, and #4 should be performed within 14 days from the time of the previously scheduled week 4, week 12, and week 24 visits, respectively.

- **Discontinuation Evaluations**:
  - Participants who are enrolled, but who do not receive vaccination #1 will be taken off study. No further evaluations are required. These participants will be replaced.
  - Premature Treatment Discontinuation Evaluations: Participants who prematurely discontinue study treatment prior to receiving vaccination #2 or #3 should remain on study (off study treatment) until the final visit. No further vaccinations should be administered. The participants should complete the premature treatment/study discontinuation valuation as specified in the SOE. Participants should complete the safety evaluations per protocol until study completion (clinical assessments, hematology, chemistries and VRC review).
  - Premature Study Discontinuation Evaluation: Participants who discontinue the study prior to the final visit should complete the premature treatment/study discontinuation evaluations as specified in the SOE.

The analysis windows will be formed around each study visit using the midpoints between adjacent weeks as cutoffs. If there are multiple evaluations within the window for a given visit, then the evaluation closest to the scheduled study week will be used, and the earlier measurement will be used if there are two measurements which are equally distant from the scheduled week.

5 Statistical Methods

5.1 Analysis of the Primary Immunogenicity Objective

The planned analysis will compare Y=sum of CEs recognized by CD4 and CD8 T cells at week 26 minus the number at baseline between the p24CE/full-length Gag DNA and placebo arm, using a two-sided 5% level Wilcoxon rank-sum test, which is anticipated to have greater statistical power than analyzing the binary version of whether Y>0, for which statistical power was based. An exact 95% confidence interval (CI) for the median of all paired differences between observations in the two arms (i.e., Hodges-Lehmann estimate) will be calculated to provide additional insight on the effect of p24CE/full-length Gag DNA vaccine. Comparisons will also be made between the Gag DNA alone vaccine arm and each of the other arms in the same manner.

The primary immunogenicity analysis for this pilot study will be per protocol, limited to participants who received all four study vaccination/placebo administrations and who did not take any medication prohibited by the study protocol on or prior to their week 26 visit. Since the amount of missing data is expected to be low in this study, the primary comparison will be based on the complete case analysis, i.e., remove records with missing data.

The following sensitivity analyses will be carried out to measure the robustness of the study findings:

- Using the stratified Wilcoxon (Van Elteren) test adjusting for the stratification factor.
- Using modified ITT approach for the primary comparison.
- Excluding the data from participants with delayed #2 and/or #3 vaccination injection due to the COVID -19 pandemic.

5.2 Analysis of the Primary Safety Objective

All participants who have been exposed to the study treatment will be included in modified intent-to-treat analysis. The primary safety outcome will be summarized separately by treatment arm, including the number and percentage of participants experiencing a primary safety outcome with an exact binomial 95% CI.

All primary safety outcome records will be listed along with AE grade, diagnosis time, vaccination time, and relationship to study treatment.

5.3 Analysis of the Secondary Objectives

The percentage of participants in each study arm with a CD4 and/or CD8 cell response to an increased number of CEs at week 26 compared to baseline will be presented along with an exact binomial 95% CI. Pairwise comparisons among study arms will be conducted using Fisher's exact test. Similar analyses will be performed for CD4 and CD8 cell responses separately.

The absolute change in total magnitude of CD4 T cell responses against each CE added together among study arms will be compared using the two-sided Wilcoxon rank-sum test. The analysis for CD8 T cell responses will be conducted in the same manner. All statistical tests will be two-sided at the 5% nominal level of significance without adjustment for multiple testing.

6 Report components

Detailed descriptions of the content of each of the following sections are given in the AIP.

1. Study entry
    a. Screening
    b. Accrual
    c. Eligibility and stratification errors
2. Baseline characteristics
3. Protocol deviations
4. Study and treatment status
5. Primary immunogenicity outcome
    a. Evaluability
    b. Primary immunogenicity outcome
    c. Sensitivity analyses
6. Safety:
    a. Primary safety outcome
    b. Deaths
    c. Overall safety
7. Secondary Outcomes