TATELO (BHP107)

Statistical Analysis Plan for Steps 1 to 3

Version 2.0

A Clinical Trial to Evaluate the Impact of Broadly Neutralizing Antibodies VRC01LS and 10-1074 on Maintenance of HIV Suppression in a Cohort of Early-Treated Children in Botswana (Dual bNAb Treatment in Children)

Protocol Version 2.0 (dated December 20, 2018)
Including LOA #1, LOA #2 and LOA #3, and CM#1, CM#2 and CM#3

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Version History

Version	Changes Made	Date Finalized
1	Original Version	10/27/2021
2	• Clarified primary safety outcome as occurring through 30 days after the last study visit (this aligns with the intended outcome measure as posted on clinicaltrials.gov). • Revised plans for exploratory analysis evaluating characteristics of participants who experienced virologic rebound during Step 2 versus participants who did not experience rebound. • Edits for clarification.	09/27/2022

1 Introduction

1.1 Purpose

This Statistical Analysis Plan (SAP) describes primary and secondary outcome measures of the Tatelo study that will be included in the primary manuscript(s), and which address, at a minimum, the primary objective of the study for Steps 1, 2 and 3 (see study design section below for description of the Steps). This SAP outlines the general statistical approaches that will be used in the analysis of these Steps of the study. It has been developed to facilitate discussion of the statistical analysis components among 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 analysis report. It also describes the results for the primary and secondary outcome measures that will be posted on ClinicalTrials.gov.

Detailed outlines of tables, figures, and coding descriptions for the Analysis Report will be included in the Analysis Implementation Plan (AIP).

Analyses will be finalized once the last participant has completed the Week 24 study visit of Step 3 and any relevant queries have been resolved. Analyses addressing the pharmacokinetic (PK) component of the primary objective of the study using PK data collected in the PK Step and in Step 1a have been undertaken previously based on a PK Step SAP.

1.2 Version History

Version 2.0 of the SAP includes the following changes compared with the previous version:

- Clarified primary safety outcome as including adverse events occurring through 30 days after the last study visit (this aligns with the intended outcome measure as posted on clinicaltrials.gov).

- Revised plans for exploratory analysis evaluating characteristics of participants who experienced virologic rebound during Step 2 versus participants who did not experience rebound.
- Minor edits for clarification.

2 Study Overview

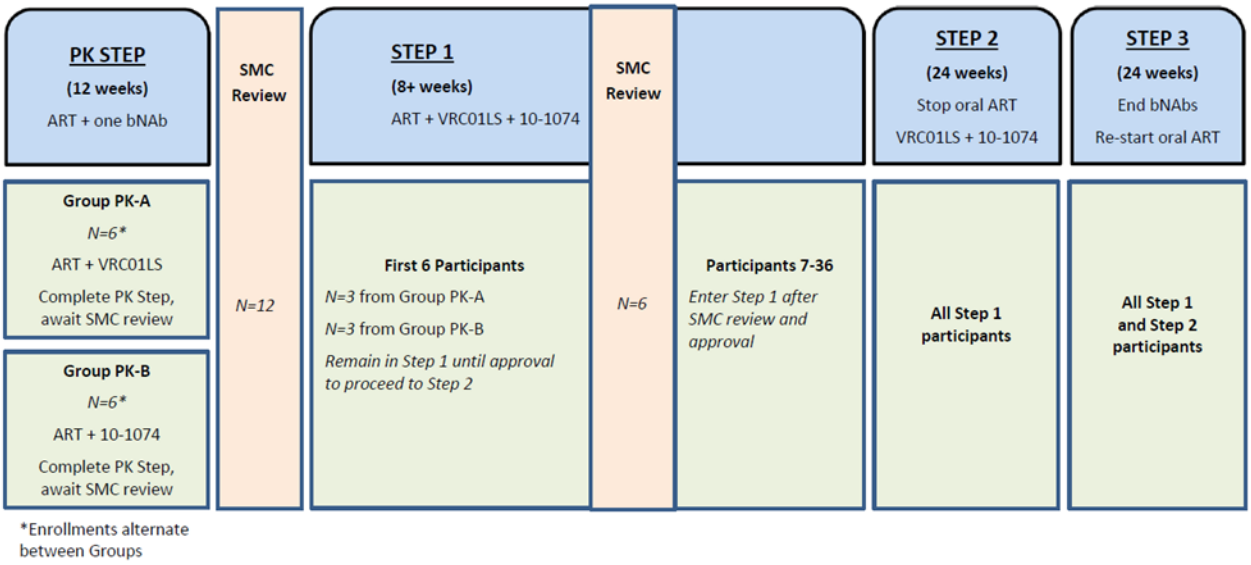
2.1 Study Design

This study is a phase I/II, multi-site clinical trial of dual treatment with two broadly neutralizing monoclonal antibodies (bNAbs), VRC01LS and 10-1074, offered to HIV-1 infected virally suppressed children. The study planned to enroll up to 40 children from two participating sites in Botswana (Gaborone and Francistown). Participants eligible for Tatelo were children with HIV who were enrolled in the Early Infant Treatment (EIT) Study (and started antiretroviral treatment [ART] shortly after birth), were at least 96 weeks old, received ART for at least 96 weeks, had HIV RNA <40 copies/mL for at least 24 weeks prior to entry, were willing to receive infusions of bNAbs, and met all other entry criteria.

There are 4 Steps in the protocol: PK Step, Step 1, Step 2, and Step 3. In the PK Step, ART was continued and 6 study participants underwent safety evaluations and PK testing following a single dose of VRC01LS (administered at Week 0, Week 4, and Week 8), and 6 study participants underwent safety evaluations and PK testing following a single dose of 10-1074 (administered at Week 0, Week 4, and Week 8).

Additional participants were eligible to be enrolled in Step 1 and were followed through Steps 1, 2 and 3. In Step 1, ART was continued and dual bNAb treatment was given. In Step 2, ART was withdrawn and dual bNAb maintenance treatment continued. In Step 3, dual bNAbs were discontinued and participants re-started ART. Figure 1 provides a schematic of the study. Note that the first six participants enrolled in Step 1 participated in an additional PK component of the study to confirm dual bNAb dosing. This is referred to as Step 1a; PK data from this step have been analyzed previously. All participants were monitored for both safety and viral rebound throughout the study.

Figure 1: Study Schematic



2.2 Study Objective Addressed in this SAP

This SAP addresses the following primary objective listed in the study protocol for Specific Aim 1 (other than the PK component of the objective for the PK Step and Step 1a which has been addressed previously), which includes the major outcomes for evaluating the effect of the study treatment. Other study objectives in the protocol will be addressed separately.

Analysis of the study objectives below will be analyzed under a proof-of-concept framework. This study does not have a control arm; however, 95% confidence intervals will be reported.

2.2.1 Primary Objective

2.2.1.1 Specific Aim 1

To conduct an interventional clinical trial to determine the safety, pharmacokinetics, dosing and antiviral efficacy of up to 24 weeks of maintenance VRC01LS and 10-1074 immunotherapy in early-treated HIV-1 infected children in Botswana.

2.3 Hypotheses

2.3.1 Specific Aim 1

VRC01LS and 10-1074 will not be associated with serious adverse events (SAEs), and median trough concentrations at the doses used will be above established target values of 200 mcg/mL and 7.5 mcg/mL respectively [note, Letter of Amendment (LOA) #2 revised PK trough target for VRC01LS to 160mcg/mL at Day 56 of Step 1]. At least 70% of early-treated low-reservoir children will remain with HIV-1 RNA <400 copies/mL after 24 weeks of receiving VRC01LS and 10-1074 maintenance.

2.4 Overview of Sample Size Considerations

Sample size considerations and estimated precision given in version 2.0 of the protocol are in relation to follow-up through the end of Step 2 of at least 20 children. With a sample size of 20 children (the minimum number expected), safety and PK evaluations will be descriptive, and there will be reasonable precision to estimate the proportion with HIV viral suppression.

2.5 Overview of Formal Interim Monitoring

An independent and external Safety Monitoring Committee (SMC) was established to review interim study data, including safety, PK and viral load. These reviews occurred at the end of the PK step, after the first 6 participants have completed at least 8 weeks of follow-up under Step 1 and then at least every 6 months, and as recommended by the SMC. Details are provided in section 13 of version 2.0 of the protocol and in LOA #3.

3 Outcome Measures

3.1 Specific Aim 1 Primary Outcome Measures

- AEs graded for severity according to Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), assessed for association with treatment, and measured through 30 days after last study visit. Clinical Research Site staff make an initial assessment of association with treatment, followed by team review. In the event of any discordance, team assessment will be used.
- HIV-1 RNA in plasma through week 24 of Step 2.

3.2 Specific Aim 1 Secondary Outcome Measures

- VRC01LS and 10-1074 concentrations in serum measured through week 32 following Step 1 entry
- Growth characteristics (sex-specific WHO standardized Z-scores for height for age and weight for age) measured at least monthly, including growth trajectories.

4 Statistical Principles

4.1 General Considerations

4.1.1 Defining baseline

Baseline is the date of initiating dual bNAbs in Step 1 (labeled as Day 0 of Step 1).

4.1.2 Analysis populations

The main analysis population is the population of children that receive any infusions in Step 1. Any children that enroll to the study but do not receive any infusions in Step 1 will be included in screening, accrual and eligibility summaries only.

4.1.3 Visit windows and schedule

The baseline visit, as defined above, is considered day 0. Baseline measurements are the last available measurement for a given parameter obtained prior to administration of this first infusion. The visit schedule is based on this day 0.

Per protocol, the scheduled visit window begins at the midpoint from the previous visit and extends to the midpoint with the next visit. During participation in Step 1 or Step 2, if a child needed to discontinue study treatment for any reason, he/she would complete final visit evaluations for the Step and enroll to Step 3.

Children enrolled to **Step 1a** (first 6 participants) had initial visits at Day 0, Day 1, Week 1, Week 4, Week 4 + 1 day, Week 5 and Week 8. Infusions occurred at Day 0, Week 4 and Week 8. Single PK draws were made at all non-infusion visits and at Week 8, while more intensive PK draws occurred on Day 0 (pre-infusion, end of infusion and 1 hour post-infusion) and Week 4 (pre-infusion, end of infusion and 1 hour post infusion at Week 4). The Step 1a follow-up extended beyond Week 8 at 2 week intervals until Week 32 (which also served as Step 2 Week 0), with infusion and single PK draws every 4 weeks. SMC review and approval of Step 1a results was required prior to starting Step 2

Step 1b (for participants enrolled after the first six participants in Step 1/SMC review) visits were scheduled at Day 0, Week 1, Week 4, Week 5 and Week 8 (Step 2 Week 0). Dual bNAbs were administered at Day 0, Week 4 and Week 8; PK measures were limited to pre-dose trough at Week 4 and Week 8 (which also served as Step 2 Week 0).

If a participant had a confirmed viral load result ≥40 copies/mL during Step 1 (either 1a or 1b), he/she would discontinue bNAbs, complete visit procedures equivalent to those outlined for Step 1 Week 8, and enter Step 3 (and not participate in Step 2).

Step 2 visits occurred every week for the first 4 weeks, then every 2 weeks through Step 2, Week 24, with dual bNAbs administered and PK trough draws every 4 weeks.

If a participant experienced a single viral rebound ≥400 copies/mL in Step 2, ART was re-started (and bNAbs discontinued). If HIV-1 RNA was ≥400 copies/mL prior to the end of Step 2, all evaluations ordinarily scheduled at Week 24 were performed immediately prior to ART re-initiation. If HIV-1 RNA was ≥40 copies/mL but <400 copies/mL, or if qualitative DNA turned from negative to positive, virologic monitoring tests were repeated weekly. All participants who did not experience viral rebound prior to week 24 entered Step 3 at the same visit as Step 2 Week 24.

In **Step 3** visits were scheduled at 4 weeks from Step 3, Day 0 (which was the same visit as Step 2 Week 24), 12 weeks, and 24 weeks. Participants who entered Step 3 due to viral rebound prior to Week 24 of Step 2 were followed for virologic monitoring visits weekly in Step 3 until HIV-1 RNA returned to <40 copies. There were no bNAbs administered or PK draws done in Step 3.

4.1.4 Adjustment for multiple comparisons

The analyses for the Steps 1-3 are descriptive and exploratory and no adjustment for multiple comparisons (e.g. across outcome measures) will be undertaken.

4.1.5 Summarizing continuous variables

Continuous variables will be summarized using minimum, lower quartile (Q1), median, upper quartile (Q3) and maximum, along with mean and standard deviation.

4.1.6 Missing data

Participants in this study have been successfully followed with demonstrated high adherence to study evaluations for at least 96 weeks before entering this study, so missing data are expected to be minimal. Missing data will be documented and the potential impact of missing data on the interpretation of results will be considered along with taking into account the specific reasons for missingness.

4.1.7 Defining SAEs

For this study a SAE, as defined by the International Conference on Harmonisation (ICH), is an AE following an infusion of a bNAb that:

- Results in death
- Is life-threatening
- Requires hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect or
- Is an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the child or may require intervention to prevent one of the other outcomes listed in the definition above.

4.2 Analysis Approaches

4.2.1. Baseline Characteristics

Baseline characteristics will be summarized using data obtained at EIT Study entry (and subsequently entered into the Tatelo Study database), as well as data collected at Tatelo Study entry on the demographics form (primarily SES information, which may have changed from the prior EIT Study collection), and also age at Tatelo entry. Data from EIT will include: maternal ART regimen at delivery; birth weight and gestational age; baseline plasma HIV RNA and PBMC DNA prior to ART start; age at EIT entry; whether the child ever rebounded after 24 weeks on ART (and length of prior virologic rebound on ART if any).

4.2.2. Primary outcomes

1. AEs will be summarized generally (any graded event) and specifically (any SAE, defined above, or any AE determined by the study team to be related to one or both bNAb

infusion). Summaries will include listings by child of all graded events and summaries by frequency of AEs by event (including all graded events and focusing on SAEs and bNAb-related AEs).

2. Cumulative proportion of children experiencing SAEs during follow-up (until 30 days after last study visit for each participant) and proportion of potentially bNAb-related events. In particular, proportions of children experiencing the following events will be summarized along with 95% exact confidence intervals (based on the Clopper-Pearson method). The denominators for the following are all participants who received at least one infusion of a bNAb in Step 1.
    a. At least one graded AE (of any grade)
    b. At least one grade 3 or higher AE
    c. At least one SAE (as defined above)
    d. At least one potentially bNAb-related AE of any grade
    e. At least one potentially bNAb-related AE grade 3 or higher
    f. At least one potentially bNAb-related SAE

3. Proportion of children, among those receiving at least one infusion of a bNAb in Step 1, who maintain HIV-1 RNA in plasma less than 400 copies/mL at least 32 weeks after initiating dual bNAb treatment (i.e., 24 weeks after withdrawal of standard ART, which is through week 24 of Step 2) will be calculated along with a 95% confidence interval (based on exact Clopper-Pearson confidence limits).

4. Repeat #3 but considering only those children who enter Step 2 and discontinue ART.

5. Repeat #3 and #4 but considering the maintenance of less than 40 copies/mL (rather than 400 copies/mL).

6. Time from Step 2, Day 0 to HIV-1 RNA ≥ 400 copies/mL will be analyzed using Kaplan-Meier methods, with censoring at last available HIV-1 RNA measurement in Step 2 if lost to follow-up or administratively at Step 2, Week 24. This will be done among all participants receiving at least one infusion of a bNAb, and among those who entered Step 2 and discontinued ART.

7. Repeat #6 but considering time to HIV-1 RNA ≥ 40 copies/mL.

8. Time to re-suppression to < 40 copies/mL (and < 400 copies/mL) will be analyzed using Kaplan-Meier methods among those who entered Step 3 because of virologic failure in Step 2; time will be measured from the day of re-starting ART in Step 3 (Step 3 Day 0).

4.2.3. Secondary outcomes

1. Summarize continuous measures of VRC01LS and 10-1074 trough concentrations in serum at Step 1, Week 4 and Week 8, and monthly during Step 2 through 24 weeks of

follow-up. Median values overall will be reported, and stratified by successes and failures in Step 2.

2. Proportion of children with trough VRC01LS and 10-1074 concentrations below defined trough values at Step 1 Week 4 and Week 8, and monthly during Step 2 through 24 weeks of follow-up (including exact confidence limits).

3. Monthly height measurements will be standardized using WHO Z-scores (for age and sex) and described at each scheduled measurement time from Step 1, Day 0 through Week 24 of Step 3. Mean changes (with 95% confidence intervals) will be obtained from Step 1, Day 0 to Step 2, Day 0 (representing change during the period of dual bNAbs + ART), from Step 2, Day 0 to Step 3, Day 0 (representing change during the period of dual bNAbs without ART), and from Step 1, Day 0 to Step 3, Week 24 (the period of complete follow-up).

4. The previous analysis will be repeated for monthly Z-score weight measurements.

5. Summarize anti-drug antibody (ADA) results for each bNAb; if warranted based on initial assessment, summarize by successes and failures in Step 2.

4.3. Exploratory objectives

1. Compare trough VRC01LS and 10-1074 levels at Step 2 entry between children who ultimately remained suppressed (<400 through Step 2) versus those who rebound to >=400 during Step 2 (restricting to children who start Step 2). Sub-analysis of levels for children who were in Step 1a (first six children) will be performed (as these six children received bNAbs in addition to ART for 32 weeks whereas subsequent children received bNAbs in addition to ART for 8 weeks).

2. Compare trough levels at end of Step 2 for children suppressed (<400 through Step 2) to levels at time of rebound to >=400 for children who rebound during Step 2 (restricting to children who start Step 2).

3. Correlate trough levels at Step 2 entry with time of rebound >=400 with censoring at 32 weeks (restricting to children who start Step 2).

4. Correlate trough levels at end of Step 2 for children suppressed (<400 through Step 2) and trough levels at time of rebound to >=400 for children who rebound during Step 2 with time to rebound (censoring at 32 weeks).

5. Evaluation of potential risk factors for viral rebound to >=400 copies/mL at/before 24 weeks in Step 2 (yes/no) (restricting to children who start Step 2). Risk factors may include demographic variables, HLA type, baseline plasma HIV RNA and PBMC DNA prior to ART (from the EIT study), PMBC DNA at Step 1 and Step 2 entry, history of prior virologic rebound on ART, duration of dual bNAb treatment (extended >8 weeks for participants in

Step 1a versus 8 weeks for other participants), trough PK values for each bNAb during Step 2 (time dependent variable), and presence of pre-defined HIV envelope sequences associated with resistance to either bNAb (earliest available in life, closest available prior to rebound, and at rebound). The extent of risk factors considered will be dependent on availability of results for these factors at the time of analysis. Analyses will compare characteristics of participants who experience rebound versus those who do not experience rebound using Fisher's Exact Test for categorical variables and Wilcoxon's Test for continuous variables).

5. Report Contents

Detailed descriptions of the content of each of the following sections will be given in the report.

1. Step 1 entry
    a. Screening (describing children from EIT by eligibility status and among those who were eligible, enrollment status)
    b. Enrollment
    c. Eligibility violations
2. Baseline characteristics
3. Protocol deviations
4. Study status
5. Study treatment (VRC01LS and 10-1074) status
6. Changes/interruptions to antiretroviral regimen during study follow-up
7. PK and ADA
8. Safety:
    a. All AEs (and potential relation to bNAbs)
    b. Grade 3+ AEs (and potential relation to bNAbs)
    c. All SAEs (and potential relation to bNAbs)
    d. Infusion reactions
    e. Deaths
9. HIV-1 RNA during study follow-up
10. Growth assessments
11. Associations of PK and virologic outcomes
12. Comparison of characteristics of participants who experience rebound during Step 2 versus those who do not experience rebound.

6. Associated Documents

6.2.2.1.1.1.1.1. Writing Team Roster

To be determined by study team.

6.2.2.1.1.1.1.2. Timetable for primary analysis and manuscript preparation

To be determined by study team.