IMPAACT 2009

Primary Statistical Analysis Plan

Version 2.0

22 October 2024

Pharmacokinetics, Feasibility, Acceptability, and Safety of Oral Pre-Exposure Prophylaxis for Primary HIV Prevention during Pregnancy and Postpartum in Adolescents and Young Women and their Infants

DAIDS ES # 30020
IND #136,735 Held by DAIDS
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Protocol Version 3.0

Created by:

Statistical and Data Analysis Center
Harvard T.H. Chan School of Public Health

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Version History

Version	Changes Made	Date Finalized
1	Original Version	January 12 2022
2.0	Added PK Component text	October 22 2024

1 Introduction

1.1 Purpose

This Primary Statistical Analysis Plan (SAP) describes the primary estimands and primary outcome measures that will address primary study objectives and interim monitoring of IMPAACT 2009. The Primary SAP includes general analytic approaches for all primary estimands, and primary outcome measures in the primary manuscript(s) or submitted to ClinicalTrials.gov (regardless of the reporting timeline). The Primary SAP facilitates discussion of the statistical analysis components among the lead study investigators and statisticians, helping them agree on the statistical analyses to be performed and presented in the primary analysis report.

The Analysis Implementation Plan (AIP) provides detailed outlines of tables, figures, and coding descriptions. A separate SAP will provide outlines of analyses for other objectives and outcome measures not included in the Primary SAP.

1.2 Version History

Not applicable; original version

2 Study Overview

2.1 Overview of Study Design

2.1.1 PK Component Study Design

The IMPAACT 2009 PK Component aims to establish drug thresholds for adequate adherence in pregnancy and postpartum when FTC/TDF is administered daily during pregnancy and in the postpartum period under directly observed conditions. All maternal participants will be administered once daily FTC/TDF. All doses will be directly observed for the 12-week duration. DBS specimens will be collected at weekly intervals for analysis.

The study population includes pregnant or postpartum women 16-24 years of age who are HIV negative and their infants: Group 1: Enrolled during singleton pregnancy at 14-24 weeks' gestation; Group 2: Enrolled postpartum within 6-12 weeks after delivery. The PK Component will enroll 40 women and their infants (20 mother-infant pairs in each Group). A total of 40 maternal participants will be recruited to obtain at least 30 evaluable maternal participants with 20 participants recruited in the antepartum group (14-24 weeks of gestation) (Group 1), and 20 recruited in the postpartum group (6-12 weeks postpartum) (Group 2). Participants agree to take once daily oral PrEP (FTC/TDF) under direct observation daily for 12 weeks. Dried blood spot (DBS) specimens will be collected at weekly intervals for analysis. Five sites across four countries (Malawi, South Africa, Uganda, and Zimbabwe) participate in the IMPAACT 2009 PK component.

A maternal participant is considered evaluable if she fulfils the following criteria; a) completes PK Component follow-up, b) has adequate documentation that PrEP doses were directly observed, c) has demonstrated adequate adherence to PrEP, defined as no more than 12 total directly observed doses missed over 12 weeks (maternal participants with more than three consecutively missed doses will be reviewed by the CMC to determine evaluability), and (d) had adequate DBS samples shipped to the PK testing laboratory, defined as no more than one missed specimen in

any given two-week interval over 12 weeks with a minimum of seven samples correctly collected, processed, handled and shipped. The rationale for this design is to collect as many weekly measurements as feasible to enable the most robust estimates, but also to allow flexibility for occasional missed visits and doses to enable practicability for participants and sites.

2.1.2 PrEP Comparison Component Study Design

The IMPAACT 2009 PrEP Comparison Component is a parallel observational cohort study. It will assess feasibility, acceptability, adherence to, and safety of PrEP during pregnancy and in the postpartum period among adolescents and young women who are HIV-negative. Women will be screened prior to 32 weeks' gestation and subsequently enrolled in two cohorts: those who choose to initiate PrEP at enrollment (Cohort 1) and those who decline (Cohort 2). Infants will be enrolled *in utero* at the same time the woman is enrolled. A randomized trial in which women were assigned to an arm that would not receive PrEP would be unethical at this time due to the overwhelming evidence supporting the efficacy of PrEP to prevent HIV infection. All women enrolled in the study will receive a standard of care package of HIV prevention services and a study-specific behavioral risk reduction intervention. Additionally, all women will receive support for antenatal, delivery and early infant care through brief motivational-interviewing informed counseling at each study visit and one-way mHealth messaging services. Women who choose to initiate PrEP will be provided PrEP as well as drug-level informed adherence support intervention.

Approximately 350 women will be enrolled to achieve at least 300 evaluable women (200 in Cohort 1 and 100 in Cohort 2) and their infants, defined as completing the week 26 postpartum visit. Women will be accrued over a 12-month period (from the date of first enrollment) and followed through 26 weeks postpartum. For each woman, the duration of follow-up is expected to range from seven to 12 months depending on gestational age at entry. For each infant, the expected duration of follow-up is approximately six months.

Regardless of cohort, all maternal participants will follow similar schedules of evaluations through the course of pregnancy and in the 26 weeks following delivery. Women in Cohort 2 may choose to initiate PrEP at any stage following study entry, and triggers for change in PrEP perceptions and acceptability over time will be recorded to better understand women's motivation to seek additional HIV prevention services. As Cohort 2 is not exposed to antiretroviral agents during pregnancy or postpartum, its unexposed time period will serve as a comparison group for estimates of incidence and prevalence of maternal and infant safety outcomes including a) pregnancy outcomes, b) maternal adverse events, and c) infant growth and development between the two exposure groups.

The primary objectives focus on women who initiate PrEP during pregnancy and continue through the first 26 weeks postpartum (Cohort 1). Adherence to PrEP will be assessed by measuring TFV-DP drug levels in dried blood spots. Participants in this arm will receive an intervention designed to provide participant-centered support, including support for PrEP use/adherence, comprised of a) two-way SMS messaging related to PrEP adherence, and b) targeted adherence counseling guided by TFV-DP drug levels.

Maternal participants who change their intent regarding PrEP will be allowed to do so. Participants in Cohort 1 who choose to stop PrEP will continue in follow-up. Maternal participants

in Cohort 2 who subsequently choose to initiate PrEP will undergo a Step Change, and will be referred to as Cohort 2/Step 2 and will follow the procedures for Cohort 1/Cohort 2 Step 2. While a formal step change will only be required when those originally declining PrEP later chose to initiate it, there is no upper limit set on the number of switches allowed for each participant. Switching will be closely monitored as part of feasibility and adherence outcomes.

There will be no randomization for this study. To monitor the balance of the maternal participants enrolled among various groups, the enrollment will be stratified by:

- Cohort: PrEP vs. No PrEP at enrollment
- Trimester of enrollment during pregnancy: 1st or 2nd trimester vs. 3rd trimester
- Country
- Age at enrollment: 16-17 years vs. 18 years of age and older

Stratifying by the above factors in this non-randomized study is for the purpose of monitoring the accrual to ensure that there are not large differences between the two cohorts (PrEP and No PrEP) in the proportion of women who are 16-17 years old, who are from a specific country, or who enroll in the 1st or 2nd (vs. 3rd) trimester of pregnancy, and to ensure a proportion of enrolled women are in the 16-17 year old age group. The team will be closely monitoring and adjusting enrollment to ensure this balance.

The primary analysis for this study will take place after follow-up of all participants in the study has concluded.

2.2 Hypotheses

2.2.1 Hypothesis for the PK Component

In the context of monitored daily administration of PrEP, a TFV-DP threshold can be determined to track adequate adherence during pregnancy and breastfeeding.

2.2.2 Hypotheses for the PrEP Comparison Component

1. At least 75% of women who opt to initiate PrEP will achieve a high level of adherence throughout pregnancy, as measured by optimal TFV-DP concentrations in DBS specimens.

2. The rate of maternal and infant adverse events (including adverse pregnancy outcomes) will be higher among mother-infant pairs in which the mother initiates PrEP during pregnancy or postpartum than in pairs in which the mother declines PrEP.

2.3 Primary and Secondary Study Objectives

2.3.1 Primary Study Objective for the PK Component

- To determine the concentration of tenofovir diphosphate (TFV-DP) associated with adequate adherence to FTC/TDF among women observed ingesting daily oral PrEP during pregnancy and postpartum.

2.3.2 Secondary Study Objective for the PK Component

- To compare TFV-DP concentrations observed in pregnant and postpartum women.

2.3.3 Primary Study Objectives for PrEP Comparison Component

This Primary SAP addresses the following primary objectives in the study protocol. Other study objectives in the protocol will be addressed in subsequent analysis plans.

- To characterize PrEP adherence among HIV-uninfected young women during pregnancy and for twenty-six weeks postpartum, when provided with enhanced adherence support through mobile technology and counseling based on observed drug levels.
- To assess the safety of FTC/TDF for PrEP during pregnancy and postpartum by comparing pregnancy outcomes and maternal and infant safety between PrEP-exposed and PrEP unexposed groups.

The Primary SAP includes objectives, estimands and outcome measures to be addressed in the primary analysis report for the PrEP Comparison Component. The analysis or monitoring reports prepared by the SDMC for IMPAACT 2009 will include monthly monitoring reports, annual and ad hoc SMC reports, and a final report to be prepared after all participants have completed their last follow up visit.

2.4 Overview of Sample Size Considerations

2.4.1 PK Component:

The PK Component will enroll a total of 40 women (20 in Group 1 and 20 in Group 2) to achieve a sample size of at least 30 evaluable participants, accounting for attrition.

2.4.2 PrEP Comparison Component:

The PrEP Comparison Component will enroll a total of 350 (233 in Cohort 1 and 117 in Cohort 2) women over a 12-month period. Women will be followed through 26 weeks postpartum. Sample size and power calculations assume a final analysis sample of 300 women (200 in Cohort 1 and 100 in Cohort 2) with follow-up through 26 weeks postpartum. The primary reason for the 2:1 enrollment into the two cohorts was to balance the need for a “No PrEP” comparison group (to provide the background rates for safety outcome measures) with the need for a large enough PrEP group to examine outcomes that would be restricted to women in that group and the periods when they were prescribed PrEP (Adherence). To achieve this sample size at the analysis phase, this study will enroll approximately 350 women to accommodate a decrease of an estimated 15% due to maternal attrition. This is an abbreviated summary of details in the study protocol; refer to the IMPAACT 2009 protocol for additional details.

2.5 Overview of Formal Interim Monitoring

PK Component and PrEP Comparison Component: The Clinical Monitoring Committee (CMC) on behalf of the Protocol Team will closely monitor study progress, safety, quality of study conduct, and participant accrual and retention based on reports that will be generated at least

monthly by the SDMC. In addition, an independent IMPAACT Study Monitoring Committee (SMC) will review this study regularly, following policies described in the IMPAACT Manual of Procedures. The composition of the SMC will include the SMC Chair; IMPAACT Chair or Vice-Chair; IMPAACT HIV Treatment Scientific Committee representative; representatives of the IMPAACT Operations Center and Statistical and Data Management Center; and representatives of NIAID and NICHD.

SMC reviews will occur at least annually and on a more frequent or ad hoc basis if any safety issues or concerns arise. Ad hoc reviews may also be triggered per the safety monitoring guidelines specified below. Based on any of its reviews, the SMC may recommend that the study proceed as currently designed, proceed with design modifications, or be discontinued. The SMC may also provide operational recommendations to help address any study implementation challenges that may be identified during their reviews.

The SMC will monitor study progress, quality of study conduct, and participant safety. The SMC will generally review the same types of data reports as the Protocol Team and CMC. For ad hoc or triggered safety reviews, more limited data may be reviewed, focusing on the events that triggered the reviews.

PrEP Comparison Component Only: Adverse events in both groups and the proportion of women with Week 12 TFV-DP drug levels below the limit of quantification in Cohort 1 (as well as decisions to initiate PrEP after study entry among women in Cohort 2) will be monitored and summaries prepared at regular intervals for review by the SMC. The following events will trigger a SMC review.

- Monitoring the proportion of women in Cohort 1 without quantifiable levels of TFV-DP at Week 12 (excluding women who have decided to stop PrEP):
    - At any site, if this proportion at Week 12 is greater than 40% after 15 maternal participants in Cohort 1 have completed that study visit and have TFV-DP specimen data available for that visit, the CMC will review the data and consult with site investigators, and make a recommendation to the SMC regarding the need for remedial actions or discontinuation of enrollment at the site. The SMC will convene to consider the CMC's recommendation.
- Monitoring participant safety outcomes (for women or infants in Cohort 1 and Cohort 2, Step 2). *Ad hoc* SMC reviews will be convened:
    - If the proportion of women experiencing Grade 3 or higher adverse events is greater than 15%, after 50 women have been enrolled
    - If the rate of adverse pregnancy outcomes is greater than 20%, after 25% of pregnancies have been completed.
    - If the proportion of livebirth infants experiencing Grade 3 or higher adverse events is greater than 15%, after 50 completed pregnancies resulting in live births.

3 Outcome Measures

3.1 Primary and Secondary Outcome Measure for PK Component

- Maternal Steady state TFV-DP concentration levels at Week 12.

3.2 Primary Outcome Measure(s) for PrEP Comparison Component

PrEP adherence:

- TFV-DP drug concentration level in Dried Blood Spots (DBS)

Maternal Adverse Events:

- Maternal Grade 3 or higher adverse events (signs, symptoms, labs, and diagnoses)
- Maternal Grade 2 or higher chemistry abnormalities

Adverse Pregnancy Outcomes:

Composite outcome of

- Spontaneous abortion (occurring at <20 weeks gestation)
- Stillbirth (occurring at ≥20 weeks gestation)
- Preterm delivery (<37 completed weeks' gestation)
- Small for gestational age (<10th percentile using **INTERGROWTH-21st** norms and ultrasound derived gestational age at delivery)

Infant Safety:

Infant Adverse Events

- Infant death within the first 26 weeks of life
- Infant Grade 3 or higher adverse events (signs, symptoms, labs, diagnoses) reported between birth and 26 weeks postpartum

Infant Growth and Development

- Infant bone mineral content based on DXA scan of the whole body (WB-BMC) at birth and lumbar spine (LS-BMC) at birth and 26 weeks postpartum
- Infant creatinine and CrCl rate at birth and 26 weeks postpartum measured by Schwartz equation
- Infant length for age z-score at birth and 26 weeks postpartum

4 Definitions

Baseline

Maternal baseline is defined as the measurement on or closest to the study entry date, and infant baseline is defined as the measurement at birth. For the PrEP Comparison Component analyses characterizing the proportion with optimal adherence to PrEP among women in Cohort 2 who subsequently decide to initiate PrEP, baseline is defined as the date initiating PrEP.

Visit Windows for Analysis

All analyses summarizing or referring to data by visit week include a visit window around the applicable study visit week. For maternal antepartum study visit weeks 4, 8, and 12 the visit window is +/- 14 days. The visit window is +/- 21 days for antepartum visit weeks 24 and 36. The Labor and Delivery/Infant birth visit may occur as soon as possible after delivery through 21 days postpartum. The visit window is +/- 21 days for the maternal and infant 6- week postpartum, 14-week postpartum, and 26-week postpartum visits.

Analysis Sets

PK Component:

- **PK Adherence Set:** All maternal participants on PrEP, including those in Group 1 and those in Group 2

PrEP Comparison Component:

- **Adherence Set:** All maternal participants on PrEP, including those in Cohort 1 and those in Cohort 2 who later decide to formally initiate PrEP. All visits/periods where maternal participants are actively prescribed PrEP.
- **Maternal Safety Set:** All maternal participants in Cohort 1 or Cohort 2, starting at enrollment
- **Infant Safety Set:** All liveborn infants in Cohort 1 or Cohort 2, starting at birth

5 General Considerations – PrEP Comparison Component

The primary analyses to address the primary objectives of the PK Component and the PrEP Comparison Component are outlined below. For a more detailed description of the analyses to be conducted, please refer to the Analysis Implementation Plan (AIP).

Categorical variables will be summarized as N (%), with corresponding 95% confidence intervals where applicable. Continuous variables will be summarized with means and standard deviations, medians and IQR (Q1, Q3), as well as min, max.

Descriptive summaries:

PK Component: We will summarize and compare demographic and clinical characteristics at enrollment between maternal PK Component Group 1 (enrolled in pregnancy) and maternal PK

Component Group 2 (enrolled postpartum). We will use Fisher's exact test to estimate differences in proportions and t tests or Wilcoxon rank-sum tests (as appropriate) for continuous measures.

PrEP Comparison Component: Entry demographic, pregnancy, pregnancy history, psychosocial, sexual behavior and partner characteristics of women in Cohort 1 and Cohort 2 will be summarized and compared using chi square tests (or Fishers exact test as appropriate) for discrete variables and t-tests or Wilcoxon tests for continuous variables.

6 Estimands and Estimation

6.1 Primary Estimand PK Component:

<table>
<tr><th colspan="2">Primary Objective 1: To determine the concentration of tenofovir diphosphate (TFV-DP) associated with adequate adherence to FTC/TDF among women observed ingesting daily oral PrEP during pregnancy and postpartum.</th></tr>
<tr><td>Estimand description</td><td>Maternal Steady state TFV-DP concentrations at Week 12</td></tr>
<tr><td>Treatment</td><td>1. Daily oral PrEP (200 mg FTC/ 300 mg TDF) during pregnancy and/or postpartum administered through direct observation.</td></tr>
<tr><th>Target population</th><th>Analysis set</th></tr>
<tr><td>Women who are pregnant and test HIV-negative, are16-24 years of age, enrolled during a singleton pregnancy at 14-24 weeks' gestation or postpartum within 6-12 weeks after delivery, and express willingness to take daily oral PrEP under direct observation for 12 weeks</td><td>PK Adherence set: Women initiating PrEP</td></tr>
<tr><th>Variable(s)</th><th>Outcome measure(s)</th></tr>
<tr><td>TFV-DP drug concentration level</td><td>1. TFV-DP drug concentration level from Dried Blood Spots (DBS) measured at week 12 among pregnant women
2. TFV-DP drug concentration level from Dried Blood Spots (DBS) measured at week 12 among postpartum women.</td></tr>
<tr><th>Handling of intercurrent events</th><th>Handling of missing data</th></tr>
</table>

• Discontinuation of PrEP: Only visits occurring prior to discontinuation of PrEP will be included. (While on treatment strategy)	Missing data could occur in the following situations: • Missing visit(s) when DBS would have been collected • Missing drug concentration results due to sample collection or processing issues. If a single specimen is missing at week 12, and a specimen is available at week 11, sensitivity analyses will be conducted including the results from the analysis of the 11-week specimen.
Population-level summary measure	Analysis approach
1. Median and IQR TFV-DP concentration levels at Week 12 in pregnant and postpartum groups.	In each of the two groups (antepartum and postpartum groups), the median and IQR of steady state concentration (Css), defined as the TFV-DP concentration observed at week 12 corresponding to 6-7 day/week adherence ("optimal adherence"), will be calculated for each group. Predicted TFV-DP concentration levels for each maternal participant will be obtained using a population PK model, and descriptive statistics will be generated. The estimated steady-state TFV-DP concentration thresholds for optimal adherence during pregnancy and postpartum will be based on the 25th percentile for 7 doses per week.

6.2 Secondary Estimand – PK Component

Secondary Objective 1: To compare TFV-DP concentrations observed in pregnant and postpartum women	
Estimand description	Maternal Steady state TFV-DP concentrations at Week 12
Treatment	1. Daily oral PrEP (200 mg FTC/ 300 mg TDF) during pregnancy and/or postpartum administered through direct observation.

Target population	Analysis set
Women who are pregnant and test HIV-negative, are 16-24 years of age, enrolled during a singleton pregnancy at 14-24 weeks' gestation or postpartum within 6-12 weeks after delivery, and express willingness to take daily oral PrEP under direct observation for 12 weeks	PK Adherence set: Women initiating PrEP
Variable(s)	Outcome measure(s)

TFV-DP drug concentration level	1. TFV-DP drug concentration level from Dried Blood Spots (DBS) measured at week 12 among pregnant women 2. TFV-DP drug concentration level from Dried Blood Spots (DBS) measured at week 12 among postpartum women.
Handling of intercurrent events	Handling of missing data
Discontinuation of PrEP: Only visits occurring prior to discontinuation of PrEP will be included. (While on treatment strategy)	Missing data could occur in the following situations: • Missing visit(s) when DBS would have been collected • Missing drug concentration results due to sample collection or processing issues. If a single specimen is missing at week 12, and a specimen is available at week 11, sensitivity analyses will be conducted including the results from the analysis of the 11-week specimen.
Population-level summary measure	Analysis approach
Difference in median TFV-DP concentration levels at Week 12 between pregnant (Group 1) and postpartum (Group 2) groups.	In each of the two groups (antepartum and postpartum groups), the median and IQR of steady state concentration (Css), defined as the TFV-DP concentration observed at week 12 corresponding to 6-7 day/week adherence ("optimal adherence"), will be calculated for each group. Differences in median Css between the antepartum and postpartum groups will be evaluated using Wilcoxon Rank Sum tests.

6.3 Primary Estimand(s) - PrEP Comparison Component

6.3.1 First Primary Estimand

Primary Objective 1: To characterize PrEP adherence among HIV-uninfected young women during pregnancy and for twenty-six weeks postpartum, when provided with enhanced adherence support through mobile technology and counseling based on observed drug levels.	
Estimand description	The probability that pregnant and postpartum HIV-uninfected women will have optimal adherence to daily oral PrEP 1) at defined timepoints; 2) during pregnancy and postpartum (through twenty-six weeks) as measured in TFV-DP concentration levels in dried blood spots during periods when women are formally prescribed PrEP

<table>
<tr><td>Treatment</td><td colspan="2">1. Daily oral PrEP (200 mg FTC/ 300 mg TDF) during pregnancy and/or postpartum.
2. Enhanced adherence support, including SMS messaging and feedback of drug levels with tailored counseling.
3. Behavioral HIV risk reduction package</td></tr>
<tr><td colspan="2">Target population</td><td>Analysis set</td></tr>
<tr><td colspan="2">Women who are pregnant and HIV-uninfected,16-24 years of age, with a confirmed singleton pregnancy of 32 weeks' gestation or less and who have access to a mobile phone, are able to receive and read SMS messages, and express willingness to take PrEP anytime between pregnancy and 26 weeks postpartum</td><td>Adherence set: Women initiating PrEP</td></tr>
<tr><td colspan="2">Variable(s)</td><td>Outcome measure(s)</td></tr>
<tr><td colspan="2">TFV-DP drug concentration level</td><td>1. TFV-DP drug concentration level from Dried Blood Spots (DBS) measured in pregnancy at 4, 8, 12, 24, 36 week study visits, Labor and Delivery, and postpartum at 6, 14, and 26 week study visits during the periods that participants are receiving PrEP. A binary indicator, "optimal adherence" will be defined as TFV-DP levels meeting or exceeding the threshold of consistent dosing of PrEP ("Green Zone") determined in the PK Component (Appendix Tables 1-2). For women who are pregnant the threshold is 600 fmol/punch if on PrEP <=6 weeks and 650 fmol/punch if >6 weeks; for women who are not pregnant the threshold is 700 fmol/punch if on PrEP <=6 weeks and 1050 fmol/punch if on PrEP >6 weeks.

2. Visits with optimal adherence according to TFV-DP level during pregnancy and during postpartum</td></tr>
<tr><td colspan="2">Handling of intercurrent events</td><td>Handling of missing data</td></tr>
<tr><td colspan="2">• Failure to start PrEP *(*in Cohort I or Cohort 2/Step 2*)* (Treatment policy strategy)
• Discontinuation of PrEP: Only visits occurring prior to discontinuation of PrEP will be included. (While on treatment strategy)
• HIV seroconversion: Only visits occurring up to the later of the date of first positive HIV test or date when PrEP is discontinued will be included. (While on treatment strategy)</td><td>Missing data could occur in the following situations:
• Missing visit(s) when adherence would have been assessed
• Missing drug concentration results due to sample collection or processing issues.
Visits without adherence data will be excluded. Sensitivity analyses will be conducted imputing "suboptimal adherence" for each missed visit/concentration result</td></tr>
<tr><td colspan="2">Population-level summary measure</td><td>Analysis approach</td></tr>
<tr><td colspan="2">1. Probability of optimal adherence according to TFV-DP levels in DBS at defined times during pregnancy and postpartum

2. Probability of optimal adherence while pregnant and up to 26 weeks postpartum</td><td>1. At each visit that TFV-DP levels are obtained, the binomial proportion of women with optimal adherence will be calculated bounded by an exact (Clopper-Pearson) 95% confidence interval.

2. The binomial proportion of visits with optimal adherence in pregnancy and postpartum periods during periods when women are</td></tr>
</table>

	formally prescribed PrEP will be calculated with exact (Clopper-Pearson) 95% confidence intervals.

Supplemental Analyses:

- Exploratory longitudinal analyses identifying baseline and time-varying predictors of optimal adherence will be conducted with multivariable regression models which account for repeated measures across time for each participant.

- Adherence (as measured in TFV-DP levels) will also be summarized as a continuous outcome measure (median, IQR and mean [SD]) at each time point noted above when drug levels are measured, and "average" drug concentrations across pregnancy and postpartum periods, will be calculated, using generalized estimating equations as appropriate. Log transformation of this outcome will be considered if the distribution is not normal.

- Other approaches to summarizing the adherence outcome will be considered. This includes an ordinal variable with categories corresponding to different dosing levels (e.g., Below limit of quantification (BLQ), Red, Yellow, Green). For this type of outcome, at each visit that drug levels are measured in DBS, descriptive analyses will summarize the proportion of women in each adherence category (refer to categories in the appendix tables 1-2). For the interim analysis the proportion of women with TFV-DP levels below the limit of quantification (BLQ) at the week 12 visit will be estimated. Additional analyses with other cutpoints (e.g., below vs. above the limit of quantification; red vs. not red) will be considered.

- Women in Cohort 2 who later decide to initiate PrEP will be included in the main estimand. In addition, baseline characteristics, and patterns of adherence and duration on PrEP will be summarized separately for women in Cohort 1 and women in Cohort 2 who later decide to initiate PrEP using the approaches outlined above.

- The following additional descriptive statistics will be calculated: The proportion of women a) with at least one visit with TFV-DP levels corresponding to optimal adherence, b) who had TFV-DP levels corresponding to optimal adherence at all visits while formally prescribed PrEP, c) who had no detectable TFV-DP levels at any visit while formally prescribed PrEP d) who formally stopped PrEP among those who had initiated, including reasons for stopping, characterizing those who stop vs continue. In addition, mean follow-up time and % of follow-up time on PrEP will be summarized.

6.3.2 Second Primary Estimand

<table>
<tr><td colspan="2">Primary Objective 2: To assess the safety of FTC/TDF for PrEP during pregnancy and postpartum by comparing pregnancy outcomes and maternal and infant safety between cohorts.</td></tr>
<tr><td>Estimand description</td><td>Incidence rate ratio of cumulative adverse events (AEs) up to 26 weeks postpartum among PrEP exposed vs. PrEP unexposed women</td></tr>
<tr><td>Treatment</td><td>1. Daily oral PrEP (200 mg FTC/ 300 mg TDF) throughout follow-up.
2. Enhanced adherence support, including SMS messaging and feedback of drug levels with tailored counseling.
3. Behavioral HIV risk reduction package</td></tr>
<tr><td>Target population</td><td>Analysis set</td></tr>
<tr><td>Women who are pregnant and HIV-uninfected,16-24 years of age, with a confirmed singleton pregnancy of 32 weeks' gestation or less and who have access to a mobile phone and are able to receive and read SMS messages</td><td>Maternal Safety Set: all enrolled maternal participants</td></tr>
<tr><td>Variable(s)</td><td>Outcome measure(s)</td></tr>
<tr><td>Occurrence of at least one of the following maternal AEs up to 26 weeks postpartum
• Grade 3 or higher adverse events
• Grade 2 or higher chemistry abnormalities</td><td>Outcome measures as defined by the variables (graded according to the NIAID DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events).
• The start of person-time for the PrEP-exposed group is study entry for Cohort 1 and Cohort 2/Step 2 entry for Cohort 2;
• The start of person-time for the PrEP unexposed group is study entry (for Cohort 2 only; person-time is censored when initiating PrEP for participants enrolled in Cohort 2/Step 2)</td></tr>
<tr><td>Handling of intercurrent events</td><td>Handling of missing data</td></tr>
<tr><td>• Failure to start PrEP (in Cohort I or Cohort 2/Step 2) (Treatment policy strategy)
• Premature discontinuation of PrEP (Treatment policy strategy)</td><td>Participants who discontinue follow-up before the 26-week postpartum visit will have their outcome determined based on data available until the time of discontinuation (i.e., a participant who discontinued follow-up without a prior AE is assumed not to have an AE had they been observed for the intended duration).</td></tr>
<tr><td>Population-level summary measure</td><td>Analysis approach</td></tr>
<tr><td>Incidence rate ratio (among PrEP exposed vs. PrEP unexposed women) of cumulative adverse events up to 26 weeks postpartum</td><td>Incidence rate ratio of cumulative AEs up to the week-26 postpartum visit (among PrEP exposed vs. PrEP unexposed women) estimated based on Poisson distribution (with 95% Wald CI).</td></tr>
</table>

Supplemental Analyses:

- Analyses will be repeated stratified by time period (i.e., antepartum (AP) and postpartum (PP), and within AP stratified by gestational age category of enrollment).

- Rates of maternal adverse events in AP and PP periods will also be calculated and compared across levels of PrEP adherence (based on TFV-DP levels in dried blood spots). Models will be fit adjusting for potential confounders.

- To compare time to first adverse event in PrEP exposed vs. PrEP unexposed women, the hazard ratio (95% CI) will be estimated with Logrank test p-value from Cox proportional hazards regression on time to first AE with PrEP exposure as a time-varying covariate.

6.3.3 Third Primary Estimand

Primary Objective 2: To assess the safety of FTC/TDF for PrEP during pregnancy and postpartum by comparing pregnancy outcomes and maternal and infant safety between cohorts.	
Estimand description	The probability of an adverse pregnancy outcome among HIV-uninfected pregnant women who are PrEP exposed compared to women who are PrEP unexposed during pregnancy.
Treatment	1. Daily oral PrEP (200 mg FTC/ 300 mg TDF) throughout follow-up. 2. Enhanced adherence support, including SMS messaging and feedback of drug levels with tailored counseling. *3.* Behavioral HIV risk reduction package

Target population	Analysis set
Women who are pregnant and HIV-uninfected,16-24 years of age, with a confirmed singleton pregnancy of 32 weeks' gestation or less and who have access to a mobile phone and are able to receive and read SMS messages	Maternal Safety Set: all enrolled maternal participants Women in Cohort 1 (all) and Cohort 2/step 2 (if enrolled to Step 2 during pregnancy) are in PrEP exposed group; Women in Cohort 2 (remained in step 1 during pregnancy) are in PrEP unexposed group.
Variable(s)	**Outcome measure(s)**
Composite adverse pregnancy outcomes of • Spontaneous abortion (occurring at <20 weeks gestation • Stillbirth (occurring at ≥20 weeks gestation) • Preterm delivery (<37 completed weeks' gestation) • Small for gestational age (<10th percentile using INTERGROWTH-21st norms and ultrasound derived gestational age at delivery)	Outcome measure as defined by the variable.
Handling of intercurrent events	**Handling of missing data**
• Failure to start PrEP *(*in Cohort I or Cohort 2/Step 2*)* (Treatment policy strategy) • Premature discontinuation ofPrEP during pregnancy (Treatment policy strategy)	Women without a pregnancy outcome will be excluded from the analysis, with an assumption that data are missing completely at random.
Population-level summary measure	**Analysis approach**
Odds ratio of an adverse pregnancy outcome among PrEP exposed women during pregnancy vs. adverse pregnancy outcomes among PrEP unexposed women in pregnancy	Odds ratio (and exact 95% CI) will be estimated and Fisher's exact test p-value will be used to evaluate differences in the proportion with at least one adverse pregnancy outcome among women who are PrEP-exposed vs. women who are PrEP unexposed during pregnancy.

Supplemental Analyses:

- Univariable and multivariable logistic regression methods will be used to evaluate associations of PrEP use and the composite outcome indicating presence vs. absence of any adverse pregnancy outcomes, controlling for potential confounders. The same analyses will be conducted for each of the pregnancy outcomes which comprise the

composite outcome separately. Analyses of small-for-gestational age births will be restricted to live births.

6.3.4 Fourth Primary Estimand

<table>
<tr><td colspan="2">Primary Objective 2: To assess the safety of FTC/TDF for PrEP during pregnancy and postpartum by comparing pregnancy outcomes and maternal and infant safety between cohorts.</td></tr>
<tr><td>Estimand description</td><td>Incidence rate ratio of cumulative adverse events up to the age 26-week visit among PrEP exposed vs. PrEP unexposed infants</td></tr>
<tr><td>Treatment</td><td>Infants exposed to the following maternal treatment in utero and/or through breastfeeding:
1. Daily oral PrEP (200 mg FTC/ 300 mg TDF) throughout follow-up.
2. Enhanced adherence support, including SMS messaging and feedback of drug levels with tailored counseling.
3. Behavioral HIV risk reduction package</td></tr>
<tr><td>Target population</td><td>Analysis set</td></tr>
<tr><td>Infants born to women who are pregnant and HIV-uninfected, 16-24 years of age, with a confirmed singleton pregnancy of 32 weeks’ gestation or less and who have access to a mobile phone and are able to receive and read SMS messages</td><td>Infant Safety Set: all enrolled infants</td></tr>
<tr><td>Variable(s)</td><td>Outcome measure(s)</td></tr>
<tr><td>Infant adverse events:
• Infant death within the first 26 weeks of life
• Infant Grade 3 or higher adverse events reported between birth and the age 26-week visit.</td><td>Outcome measures as defined by the variable (graded according to the NIAID DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events).
The start of person-time for the PrEP-exposed group is birth for Cohort 1 and birth or Cohort 2/step 2 entry (whichever is later) for Cohort 2;
The start of person-time for the PrEP unexposed group is birth (Cohort 2 participants only; person-time is censored when initiating PrEP for participants enrolled in Cohort 2/Step 2).</td></tr>
<tr><td>Handling of intercurrent events</td><td>Handling of missing data</td></tr>
<tr><td>• Mother’s failing to start PrEP (in Cohort I or Cohort 2/Step 2) (Treatment policy strategy)
• Mother’s discontinuation of PrEP (Treatment policy strategy)</td><td>Participants who discontinue follow-up before the age 26-week visit will have their outcome determined based on data available until the time of discontinuation (i.e., a participant who discontinued follow-up without a prior AE is assumed not to have an AE had they been observed for the intended duration).</td></tr>
<tr><td>Population-level summary measure</td><td>Analysis approach</td></tr>
<tr><td>• Incidence rate ratio (among PrEP exposed vs. PrEP unexposed infants) of cumulative adverse events up to the age 26-week visit</td><td>• Incidence rate ratio of cumulative adverse events up to the age 26-week visit (among PrEP exposed vs. PrEP unexposed infants) estimated based on Poisson distribution (with 95% Wald CI).</td></tr>
</table>

Supplemental Analyses:

- Analyses will be repeated stratifying by time of infant drug exposure (i.e., antepartum (AP) and postpartum (PP), and within AP stratified by gestational age category of maternal enrollment).

- Rates of infant adverse events will also be calculated and compared across levels of maternal PrEP adherence (based on TFV-DP levels in dried blood spots). Models will be fit adjusting for potential confounders.

- To compare time to first infant adverse event in PrEP exposed vs. PrEP unexposed infants, the hazard ratio (95% CI) will be estimated with Logrank test p-value from Cox proportional hazards regression on time to first AE with PrEP exposure as a time-varying covariate.

6.3.5 Fifth Primary Estimand

<table>
<tr><th colspan="2">Primary Objective 2: To assess the safety of FTC/TDF for PrEP during pregnancy and postpartum by comparing pregnancy outcomes and maternal and infant safety between cohorts.</th></tr>
<tr><td>Estimand description</td><td>Difference in mean infant growth and development outcomes at birth and age 26 weeks among PrEP exposed and PrEP unexposed infants;</td></tr>
<tr><td>Treatment</td><td>Infants exposed to the following maternal treatment in utero and/or through breastfeeding:
1. Daily oral PrEP (200 mg FTC/ 300 mg TDF) throughout follow-up.
2. Enhanced adherence support, including SMS messaging and feedback of drug levels with tailored counseling.
3. Behavioral HIV risk reduction package</td></tr>
<tr><th>Target population</th><th>Analysis set</th></tr>
<tr><td>Infants born to women who are pregnant and HIV-uninfected, 16-24 years of age, with a confirmed singleton pregnancy of 32 weeks' gestation or less and who have access to a mobile phone and are able to receive and read SMS messages</td><td>Infant Safety Set: growth and development

Infants of women who have ever used PrEP (in Cohort 1 or Cohort 2 Step 2) will be considered PrEP exposed. Infants of women who have never used PrEP will be considered PrEP unexposed. If PrEP exposure occurs prior to the outcome measurement of interest then the infant will be considered PrEP exposed.</td></tr>
<tr><th>Variable(s)</th><th>Outcome measure(s)</th></tr>
<tr><td>Infant growth and development outcomes</td><td>The following will be calculated to evaluate infant safety outcomes:
• Infant bone mineral content (grams) by dual energy X-ray absorptiometry (DXA) scan of the whole body (WB-BMC) at birth and lumbar spine (LS-BMC) at birth and 26 weeks
• Infant creatinine and CrCl rate measured by Schwartz equation at birth and at 26 weeks
• Infant length-for-age z-score at birth and at 26 weeks
The week 26 measurement will be at the Week 26 study visit</td></tr>
</table>

Handling of intercurrent events	Handling of missing data
• Mother's failure to start PrEP *(*in Cohort I or Cohort 2/Step 2*)* (Treatment policy strategy) • Mother's discontinuation of PrEP(Treatment policy strategy)	Sensitivity analysis: for infants discontinuing study prior to the 26 weeks of age, use the last value carried forward for the 26 weeks visit.
Population-level summary measure	**Analysis approach**
Difference in mean infant growth and development outcomes between PrEP exposed (in Cohort 1 and Cohort 2 step 2) and PrEP unexposed infants (infants in Cohort 2) at birth and 26 weeks of age	Means will be estimated and compared between PrEP-exposed and PrEP unexposed infants using two sample t-tests at each time point of evaluation, or Wilcoxon rank sum tests as appropriate to evaluate differences in these endpoints between PrEP-exposed and PrEP unexposed infants.

7. Report Contents

An overview of the contents of the final analysis report for the PrEP Comparison Component is outlined below. Additional details of the final analysis report contents are provided in the AIP.

- CONSORT diagram
- Study entry:
  - Screening
  - Accrual
  - Eligibility
- Baseline characteristics (reported in ClinicalTrials.gov)
  - Maternal characteristics at baseline
  - Infant characteristics at birth
- Study retention (reported in ClinicalTrials.gov)
  - By Cohort and study visit
- Treatment status:
  - By Cohort (Cohort 1-PrEP, Cohort 2-No PrEP)
  - Frequency and Timing of switching from No PreP to PrEP (i.e., Cohort 2 step change)
- PrEP discontinuation, and PrEP restart (if applicable)
  - PrEP discontinuation reasons
- PrEP adherence:
  - N (%) with optimal adherence at each visit, overall, during pregnancy, and postpartum, and by gestational age group at enrollment
  - Number and timing of visits missed when assessment of PrEP adherence was expected
- Maternal safety outcomes
  - By PrEP exposure
  - During pregnancy and postpartum
- Pregnancy Outcomes
  - Overall and by PrEP exposure
- Infant safety outcomes
  - Overall, by PrEP exposure

8 Associated Documents

Attachment 1: Timetable for Primary Analysis and Manuscript

Event	Responsible party	Weeks from primary completion date (PCD)
Primary completion date (PCD)		PCD
Clinical data entry termination	Sites	PCD + 4 weeks
Initial database clean-up complete, including - DAERS reconciliation complete - TSDV complete	PDM and LDM	PCD + 12 weeks
Clinical database closure/freeze complete/eCRF sign-off by site investigators	PDM and Sites	PCD + 15 weeks
SDTM ARMCD populated	SDTM Specialist	PCD + 16 weeks
Complete SDTM on production server	SDTM Specialist	PCD + 16 weeks
Clinical database lock and primary laboratory database lock	Chief Data Manager and LDM Leadership	PCD + 17 weeks
Final SDTM on production server	SDTM Specialist	PCD + 18 weeks
Primary analysis report to Protocol Chairs/Writing Team	Protocol statisticians	PCD + 22 weeks

9 Appendix: Adherence Interpretations

The following Appendix Tables are adherence interpretations and categories based on TFV-DP Concentration (fmol/punch) in Dried Blood Spots (DBS) from the PK Component.

Appendix Table 1: Pregnant Women TFV-DP Concentrations in DBS at <=6 Weeks on PrEP and >6 Weeks and Beyond

Interpretation	≤ 6 Weeks on PrEP	> 6 Weeks on PrEP and Beyond
Green Zone Consistent with Highest levels of protection	≥600	≥650
Yellow Zone Consistent with some but incomplete levels of protection	150-599	200-649
Red Zone Consistent with low to no levels of protection	<150	<200

Appendix Table 2: Postpartum Women TFV-DP concentrations in DBS at <=6 Weeks on PrEP and >6 Weeks and Beyond

Interpretation	≤ 6 Weeks on PrEP	> 6 Weeks on PrEP and Beyond
Green Zone Consistent with Highest levels of protection	≥700	≥1050
Yellow Zone Consistent with some but incomplete levels of protection	200-699	300-1049
Red Zone Consistent with low to no levels of protection	<200	<300