SCHARP
Statistical Center for HIV/AIDS Research and Prevention

STATISTICAL ANALYSIS PLAN

Phase 3B, Randomized, Open-Label, Safety, and Drug Detection Study of Dapivirine Vaginal Ring and Oral TRUVADA® in Breastfeeding Mother-Infant Pairs

MTN-043

Effective Date: November 4, 2022

Version: 2.0

STATISTICAL ANALYSIS PLAN

Protocol Name:	*Phase 3B, Randomized, Open-Label, Safety, and Drug Detection Study of Dapivirine Vaginal Ring and Oral TRUVADA® in Breastfeeding Mother-infant Pairs*
Protocol Number:	*MTN-043*
Author(s):	*Barbra Richardson, Protocol Statistician* *Holly Gundacker, Statistical Research Associate*
Version:	*2.0*

Author(s):

Barbra Richardson, *Protocol Statistician*	*(see attached email approval)* Signature	*(see attached email approval)* Date:
Holly Gundacker *Statistical Research Associate*	(see attached email approval) Signature	(see attached email approval) Date:

Approvals:

Print name: Job Title:	Signature	Date: DD/MM/YYYY

TABLE OF CONTENTS

1. LIST OF ABBREVIATIONS AND ACRONYMS

A list of abbreviations used in the SAP.

Term/Abbreviation	Definition
AE	Adverse Event
AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
AUC	Area Under the concentration Curve
CASI	Computer assisted self-interview
CBC	Complete Blood Count
CI	Confidence Interval
C_{max}	Maximum Concentration
CRF	Case Report Form
CV	Coefficient of Variation
CVF	Cervicovaginal Fluid
CT	Chlamydia trachomatis
DAIDS	Division of AIDS
DBS	Dried blood spot
DPV	Dapivirine
EAE	Expedited Adverse Event
eCRF	Electronic Case Report Form
FTC	Emtricitabine
FTC-TP	Emtricitabine triphosphate
GC	Neisseria gonorrhoeae
GM	Geometric Mean
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
IDI	In-depth Interview
IQR	Interquartile Range
IRB/EC	Institutional Review Board/ethics committee
IVR	Intravaginal Ring
ITT	Intent to treat
LLOQ	Lower limit of quantification

MCV	Mean corpuscular volume
MedDRA	Medical dictionary for regulatory activities
MTN	Microbicide Trials Network
NAAT	Nucleic acid amplification test
PD	Pharmacodynamics
PEP	Post-exposure prophylaxis
PK	Pharmacokinetics
PrEP	Pre-exposure prophylaxis
PUEV	Product use end visit
SA	South Africa
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCHARP	Statistical Center for HIV/AIDS Research and Prevention
SD	Standard Deviation
SDMC	Statistical Data Management Center
SMC	Study Monitoring Committee
SRA	Statistical Research Associate
STIs	Sexually Transmitted Infections
TDF	Tenofovir disoproxil fumarate
TFV	Tenofovir
TFV-DP	Tenofovir diphosphate
T_{max}	Time to Reach the Maximum Concentration
TV	Trichomonas vaginalis
VR	Vaginal ring
WBC	White blood cell

2. INTRODUCTION

This Statistical Analysis Plan (SAP) document covers the final analysis of MTN-043. The analysis of primary and secondary endpoints with supporting summaries are included. Some selected exploratory endpoint analyses are also be included.

2.1 General Design Considerations

Short Title: B-PROTECTED: Mother-Infant Pair Study of Dapivirine Ring and PrEP in Breastfeeding

Clinical Phase: Phase 3B

IND Sponsor: DAIDS

Funders:	Division of AIDS, NIAID, NIMH, NICHD, US NIH
Protocol Chair:	Maxensia Owor, MBChB, MMed, MPH
Protocol Co-Chairs:	Lisa Noguchi, PhD, CNM Jennifer Balkus, PhD, MPH
Sample Size:	Approximately 200 mother-infant pairs
Study Population:	Healthy, HIV-uninfected breastfeeding women and their healthy infants between 6 and 12 weeks old (inclusive) at the time of enrollment
Study Sites:	• Blantyre, Malawi • Kampala, Uganda (MU-JHU) • Shandukani, South Africa (Wits RHI) • Zengeza, Zimbabwe
Study Hypotheses:	• Maternal exposure to study products will be safe for mothers and their breastfeeding infants. • Dapivirine (DPV) will be detectable at low levels in breast milk of participant mothers using the vaginal ring (VR). • Emtricitabine (FTC) and tenofovir (TFV) will be detectable at low levels in breast milk of participant mothers taking Truvada. • DPV will be detectable in the blood of some breastfeeding infants. • Emtricitabine triphosphate (FTC-TP) and/or tenofovir diphosphate (TFV-DP) will be detectable in the blood of some breastfeeding infants.
Study Design:	Phase 3B, randomized, open-label, multi-site, mother-infant pair safety and drug detection study, with 12 weeks of planned study product exposure to either DPV VR (25 mg) or oral Truvada tablet (200 mg emtricitabine [FTC]/300 mg tenofovir disoproxil fumarate [TDF]).
Study Duration:	Each enrolled mother-infant pair will be followed for approximately three and a half months (14 weeks). Note: If a mother seroconverts on study, her infant will have an additional visit, 12 weeks after seroconversion is diagnosed, for additional HIV testing.
Study Products:	• Silicone elastomer matrix VR containing 25 mg of DPV • Oral tablets (Truvada) containing 200 mg FTC/300 mg TDF
Study Regimen:	Mother-infant pairs will be randomized to the above study products in a 3:1 ratio (VR: tablet). For mothers randomized to the DPV VR, the VR will be worn continuously for approximately one month (4 weeks), to be replaced monthly (4 weeks) for approximately three months (12 weeks). Mothers using Truvada tablet will take one tablet by mouth daily for approximately three months (12 weeks).

.

2.2 Study Objectives and Endpoints

Primary Objectives:

Maternal Safety Outcomes: To describe the maternal safety profile associated with study product exposure during breastfeeding in both study arms.

Infant Safety Outcomes: To describe the infant safety profile associated with study product exposure during breastfeeding in both study arms.

Drug Detection: To summarize the frequency of study drug detection and concentration of study drug(s) in mothers and their breastfeeding infants.

Primary Endpoints:

Maternal safety (composite)

- All serious adverse events (SAEs) including maternal deaths in both study arms
- All Grade 3 or higher adverse events (AEs) as defined by the *Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1, July 2017* and/or *Addendum 1 (Female Genital Grading Table for Use in Microbicide Studies [Dated November 2007])* in both study arms

Infant safety (composite)

- All SAEs including infant deaths in both study arms
- All Grade 3 or higher AEs as defined by the *Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1, July 2017* in both study arms

Drug Detection

- Maternal plasma DPV concentrations
- Maternal blood FTC-TP and TFV-DP concentrations
- Maternal breast milk DPV concentrations
- Maternal breast milk FTC and TFV concentrations
- Infant plasma DPV concentrations
- Infant blood FTC-TP and TFV-DP concentrations

Secondary Objectives:

Adherence: To characterize adherence to open-label use of the DPV VR (25 mg) and oral Truvada in breastfeeding women.

Acceptability: To characterize acceptability of open -label use of the DPV VR (25 mg) and oral Truvada in breastfeeding women.

Secondary Endpoints:

Adherence

- Participant report of frequency of study product use (e.g., missed doses for oral Truvada and VR removal/expulsions [voluntary and involuntary] and duration without VR in vagina)
- Residual drug levels in returned VRs
- Maternal plasma DPV concentrations
- Maternal blood FTC-TP and TFV-DP concentrations

Acceptability

- Self-reported attitudes about study product attributes and willingness to use their assigned study product during breastfeeding in the future
- Proportion of participants who find their study product to be at least as acceptable as other HIV prevention methods

2.3 Randomization

Participants were randomized in a 3:1 ratio (VR:tablet) to the two arms of the study stratified by study site. The randomization scheme was generated and maintained by the MTN SDMC.

2.4 Blinding

MTN-043 is an open label study. The study product assignments were not blinded.

2.5 Sample Size and Power

The target sample size was 200 mother-infant pairs randomized 3:1 to vaginal ring vs oral Truvada. A total of 197 mother-infant pairs were enrolled. The sample size is based upon feasibility, anticipated low drug concentrations, and the likelihood of a favorable safety profile. The statistical properties of the study are described using the probability of observed safety events defined in the primary endpoint. The probabilities of observing a safety event for various “true” event rates were calculated. For example, if the true safety event rate was 5%, the probability of observing zero safety events is 0.05% for the cohort size of 150 participants (DPV VR) and is 7.69% for cohort size of 50 participants (Truvada). An alternative way of describing the statistical properties is in terms of 95% confidence interval. For example, if none of the participants experience a safety event, the 95% exact 2-sided upper confidence bound for the true event rate is 2.43% for participant receiving the VR regimen and is 7.11% for participants receiving Truvada. The properties above also inform the hypotheses around drug detection where number of events is the number of participants with detectable drug levels. Further details of the statistical properties for this study are described in section 10.5 of the protocol.

3. GENERAL DATA ANALYSIS CONSIDERATIONS

3.1 Analysis Set(s)

Following the intent to treat (ITT) approach, participants will be classified by their randomized study arm regardless of the study product received. Participants found to be ineligible post-enrollment will be excluded from all analyses. For the primary safety endpoints (maternal safety, infant safety) participants enrolled and who received at least one dose of study product will be included. All participants with at least one post-enrollment drug concentration result will be included in the primary analysis for drug detection.

For analysis of drug concentrations, a secondary analysis will be conducted including only visits in which a participant has been exposed to the study product and excluding visits of mothers or infants with mothers terminating the study early or on product hold.

For the secondary endpoint of adherence, all mothers will be included. However, reasons for study product non-use from all mothers will be tabulated. A secondary sensitivity analysis will be conducted including only mothers with drug concentration results for at least one visit after Enrollment.

For the secondary endpoint of acceptability, all mothers with non-missing responses for the acceptability and preference questions (#1, #2, #5, #6 and #7) on the Behavioral Assessment – Month 3 Follow Up

eCRF will be included. Mothers with some missing responses to the identified questions will be included in each part of the acceptability analysis as appropriate.

3.2 Statistical Analysis Issues

In the spirit of the ITT approach, data collected outside of a participant's visit window will be included in the analysis.

Due to the occurrence of a pandemic during the conduct of this study, there may be more than expected missing data. Any missing data will be tabulated as a separate category when reporting data summaries. If a substantial amount of endpoint data is missing (e.g., follow up data missing in at least 10% of participants), then secondary sensitivity analyses of the endpoints will be conducted using methods that relax the missing completely at random assumption to a missing at random assumption. For a univariate binary and quantitative outcome, respectively, a generalized linear model with a binomial or Normal error distribution will be used for estimation and testing. These secondary sensitivity analyses will include imputing the data under the most extreme scenarios of information missingness, such as assuming participants with missing data has an extreme value of the missing variable.

4. INTERIM ANALYSIS AND DATA MONITORING COMMITTEE

No interim analysis was planned or performed for the MTN-043 study.

5. GENERAL ANALYSIS METHODS

Descriptive statistics will be used to assess group characteristics or differences by study arm. Categorical variables will be summarized using the number and percent in each category. The Pearson-Clopper method[1] will be used to obtain a 95% confidence interval (CI) on the proportion of binary variables where applicable. Continuous variables will be summarized using the number of non-missing values, mean, median, standard deviation, quartiles and range (minimum, maximum) unless otherwise indicated.

The study arms will be identified and formatted as "DPV vaginal ring" and "Truvada oral tablet".

When use of formal testing to assess differences is required, the following methods will be used unless otherwise specified: Fisher's exact test or logistical linear regression for categorical response variable; t-test or linear regression for continuous outcomes. Hypothesis tests for the primary and secondary endpoints comparing the two study arms will have a two-sided significance level of 5%.

Within study arm assessment of the change from baseline measurement to a follow up measurement will be analyzed using McNemar's test (for categorical response variables) or the paired t-test or Wilcoxon signed-rank test (for continuous variables).

To assess the adequacy of the randomization, participants in each of the two arms will be compared for baseline characteristics including demographics and laboratory measurements using descriptive statistics. No formal statistical comparisons will be performed.

As mentioned previously, there may be more than the usual amount of missing data in this study. Appropriate methods will be used to characterize and adjust for missingness as described in Section 3.2 above.

6. TRIAL PARTICIPANT DISPOSITION

6.1 Disposition of Participants

Enrollment of participants took place at four sites in Africa: Blantyre, Malawi; Zengeza Zimbabwe; Kampala, Uganda; Shandukani, South Africa (Johannesburg, SA). The mothers and infants screened and the reasons for those not enrolled will be summarized in tables by study site. The mother/infant pairs screened and enrolled by month will be summarized by study site. In addition, a consort diagram will describe the screening, eligibility, enrollment, randomization, and discontinuations from study participation. The consort diagram (or a separate table as appropriate) will describe the number of the participants with the minimum required data (from assays or eCRFs as described in Section 3.1 above) for inclusion in the primary and secondary analyses.

6.2 Retention

For each visit, a participant is expected to have returned to the site clinic within a protocol-specified visit window based on the enrollment visit date. The proportion of mothers and infants retained at a scheduled visit is obtained by dividing the number of participants who have completed the visit by the number of participants expected for that visit. Tables will be presented that show by visit the number and percentage of expected, retained, and missed visits in addition to mothers and infants lost-to-follow up or terminated early from the study by site and by arm. No formal statistical testing will be conducted.

6.3 Study and Study Product Discontinuation

The number and percentage of mothers and infants who completed the study, along with the number and percentages of participants who did not complete the study for reasons collected on the Study Termination eCRF will be presented in tables by site and by arm. Reasons for not completing the study include the following: death, unwilling or unable to comply with required study procedures, lost to follow up, investigator decision, refusal of further study product use, early study closure, protocol deviation, adverse event (mother and/or infant), withdrawal of consent by mother (or for the infant by the infant's mother), study terminated by sponsor, and other. No formal statistical testing will be conducted.

The number and percentage of mothers who completed the study product regimen, along with the number and percentages of mothers who did not complete the study product as specified per protocol for the reasons collected on the Discontinuation of Study Product eCRF will be presented in tables by site and by arm. Reasons for not completing the study product regimen per protocol include the following: death, refusal of further participation, unwilling or unable to comply with required study procedures, lost to follow up, investigator decision, refusal of further study product use, HIV infection, early study closure, protocol deviation, adverse event (mother or infant), withdrawal of consent by participant, study terminated by sponsor, acquisition of hepatitis B infection (for Truvada group only), allergic reaction to the study product, reported use of PrEP for HIV prevention outside of the study, reported use of PEP for potential HIV exposure, non-therapeutic infection drug use, pregnancy, and other. No formal statistical testing will be conducted.

6.4 Visit Adherence: Completion of Procedures

Completion of the following required and expected procedures for mothers will be evaluated: 1) social harms; 2) baseline behavioral assessment; 3) follow up behavioral assessment; 4) 3 month behavioral assessment; 5) in-depth interview (IDI); 6) baseline infant feeding assessment; 7) follow up infant feeding assessment; 8) use of vaginal products; 9) physical exam; 10) pelvic exam; 11) pregnancy test; 12) HIV-1 test; 13) hepatitis B surface antigen test; 14) blood serum chemistry; 15) hematology; 16) syphilis serology; 17) NAAT (pelvic) for GC/CT/TV; 18) plasma for archive; 19) dried blood spot (DBS) for TFV-DP and FTC-TP drug levels (for Truvada group only); 20) plasma for DPV drug levels (DPV VR group only); 21) breastmilk for drug levels; 22) vaginal swab for microbiota; 23) vaginal gram stain; 24) vaginal swab for biomarkers; 25) used vaginal ring (DPV VR group only).

Completion of the following required and expected procedures for infants will be evaluated: 1) physical exam; 2) infant ages and stages assessment; 3) DBS for TFV-DP and FTC-TP drug levels (for infants of mothers in Truvada group only); 4) plasma for DPV drug levels (for infants of mothers in DPV VR group only).

Tables displaying the number and percentages of participant-visits in which these procedures were completed, by arm and by site, will be presented. No formal statistical testing will be conducted.

6.5 Protocol Deviations

Protocol deviations, as collected in the Protocol Deviations Log eCRF, will be reported. The number and percentage of participants with protocol deviations, along with the number and percentage of participants experiencing each type of protocol deviation, will be tabulated by site and by arm. Type of deviations include: inappropriate enrollment, failure to follow randomization or blinding procedures, study product management deviation, study product dispensing error, study product use/non-use deviation, study product sharing, study product not returned, conduct of non-protocol procedure, improper AE/EAE follow up, unreported AE or EAE, breach of confidentiality, physical assessment deviation, lab assessment deviation, mishandled lab specimen, staff performing duties that they are not qualified to perform, questionnaire administration deviation, counseling deviation, use of non-IRB/EC-approved materials, use of excluded concomitant medications, devices, or non-study products, informed consent process deviation, visit completed outside of window, and other.

Additionally, a listing with all the reported protocol deviations will be presented. The listing will include the type of deviation, the deviation date, the date that the site was aware of the deviation, whether the deviation was reported to the local IRB/EC, whether the deviation was reported to DAIDS, the description of the deviation, the steps taken to address the deviation, and the steps taken to prevent future occurrences. No formal statistical testing will be conducted.

7. BASELINE DATA

Baseline characteristics of all enrolled participants will be compared by their study product assignment as randomized and by site. No formal statistical comparisons will be performed for any of the baseline results described below.

7.1 Demographics

Baseline demographic characteristics will include ethnic group or tribe, age and age category, sex at birth (for infants), number of sex partners in participant's lifetime (for mothers), and whether the participant has a primary sex partner (for mothers). Tables for mothers and infants will include summary statistics appropriate for the measurement scale by site and by arm.

7.2 Pregnancy History

The mothers' history of prior pregnancies as collected on the Pregnancy History eCRF at the Screening Visit will be presented in tables by arm and by site. Summary statistics appropriate for the measurement scale as described in Section 5 will be presented for the following: number of full term live births (>=37 weeks), number of premature live births (less than 37 weeks), number of spontaneous fetal deaths and/or still births (>=20 weeks), number of spontaneous abortions (less than 20 weeks), number of therapeutic/elective abortions, number of ectopic pregnancies, and whether the participant has a history of pregnancy complications or fetal/infant congenital anomalies (yes/no). In addition, the total number of prior pregnancy outcomes will be calculated by summing these numbers of reported births and abortions from the categories above.

7.3 Pelvic Exam

The mothers' baseline results from the pelvic exam as collected on the Pelvic Exam eCRF at Screening and Enrollment will be presented in tables by arm and by site. Number and percentage of participant-visits for which any abnormal finding was reported will be presented, as well as the number and percentage of specific types of pelvic exam findings.

7.4 Sexually Transmitted Infections

The mothers' baseline sexually transmitted infections (STIs) testing results as collected on the STI Test Results eCRF at Screening (and if indicated at Enrollment) will be presented in tables by arm and by site. Summaries (as described in section 5) of testing results will be presented for the following: pH, bacterial vaginosis, wet prep for candidiasis (buds and/or hyphae[yeast]), trichomonas (TV), syphilis screening and confirmatory test, urine NAAT for gonorrhea (GC), chlamydia (*C. trachomatis*, CT), and hepatitis B surface antigen.

8. PRIMARY ENDPOINT ANALYSES

Analysis (see Section 3.1) will be performed to summarize the frequency of primary endpoints by study arm. Consistent with the primary objectives to describe the safety profile and drug detection in each arm, the number and the percentages of participant experiencing each primary endpoint will be tabulated by study arm. Each participant will contribute once in each category (i.e. only for highest severity AE for each participant) for calculation of event rates. An exact binomial confidence interval (CI) will be calculated for each primary endpoint.

8.1 Maternal Adverse Events

The number and percentage of mothers with 1) serious adverse events (SAEs) and 2) Grade 3 or higher AEs will be tabulated by study arm. Each participant will contribute once for the calculation of the event rates. The estimated rates with exact binomial 95% confidence intervals (CIs) will be calculated by study arm.

See section 10.1 for a description of additional summaries of AEs.

8.2 Infant Adverse Events

The number and percentage of infants with 1) serious adverse events (SAEs) and 2) Grade 3 or higher AEs will be tabulated by study arm. Each participant will contribute once for the calculation of the event rates. The estimated rates with exact binomial 95% CIs will be calculated by study arm.

8.3 Drug Detection

The proportion of participants with detectable drug levels in each study arm for each sample type will be summarized using descriptive statistics in tables by arm and by site. Participants with a drug level for the assigned study product arm above the lower limit of quantification (LLOQ) for any visit after Enrollment will be considered to have a detectable drug level; otherwise, the participant will be considered to have an undetectable drug level.

Drug concentration levels will be summarized using geometric mean, geometric coefficient of variation (CV), arithmetic mean, standard deviation (SD), median, interquartile range (IQR), and range (minimum, maximum). In addition, the number and percentage of participants with detectable and undetectable drug levels will be reported. The CV will be calculated as 100 x standard deviation (SD) / Mean of the concentration values. Figures for concentration values will be presented using the log scale.

For drug concentrations that fall below the corresponding assay's LLOQ, we will use a value equivalent to half the LLOQ. The number of days to each sample collection will be calculated using the Enrollment date and the date of sample collection as reported in the drug concentrations dataset from the SCHARP Lab Data Management group. For mothers, the time points for drug concentration sample collections are on Visits 3-8 (Week 1 to 2 weeks after product use end visit [PUEV]). Dried blood spot (DBS) samples are collected from mothers at Visit 2, Enrollment in addition to Visits 3-8. For infants, the time points for drug concentration sample collections are on Visits 4-8 (Week 2 to 2 weeks after PUEV).

For the mothers on the DPV VR arm, concentrations of DPV from plasma and breast milk will be reported for each visit in a table by study site. For infants of mothers on the DPV VR arm, concentrations of DPV from plasma will be reported for each visit in a table by study site. Box plots with median DPV levels of plasma concentrations for mothers and infants and of breast milk concentrations for mothers will be presented over the study visit timepoints.

For mothers on the Truvada arm, concentrations of FTC-TP and TFV-DP from DBS and FTC and TFV from breast milk will be reported for each visit in a table by study site. For infants of mothers on the Truvada arm, concentrations of FTC-TP and TFV-DP from DBS will be reported for each visit in a table by study site. Box plots with median drug concentration levels of DBS concentrations for mothers and infants and of breast milk concentrations for mothers will be presented over the study visit timepoints.

9. SECONDARY ENDPOINT ANALYSES

9.1 Adherence

For the secondary endpoint of adherence, all mothers will be included. A secondary sensitivity analysis will be conducted including only mothers with drug concentration results for at least one visit after Enrollment.

For women randomized to the VR arm, a combination of measures will be used to characterize use of study product. In addition to the summary of maternal plasma DPV concentrations as described above in section 8.3, the residual DPV levels in the returned used rings will be summarized by study visit.

For women randomized to Truvada, maternal FTC-TP and TFV-DP concentrations from DBS sample collections, as described above in section 8.3, will be used to quantify study product use.

Cut-point Analyses
Drug levels will be summarized (number and percent) by categories defined by the following cut-points for each product. Because DPV ring release rates have not been verified against directly observed use, the only comparable category for the two products is no use.

DPV ring:
No use (rate <= 0.9mg/month)
Some release (0.9mg/month < rate <= 4.0mg/month)
High release (rate > 4.0mg/month)

Truvada:
No use (<16.6fmol/punch)
Some use (16.6-699fmol/punch)
High use (>=700fmol/punch)

DPV release rate is defined as the amount of DPV released from the ring divided by the duration of time during which the participant had the ring. The amount of DPV release is calculated by subtracting the

amount of residual DPV in a returned ring from the amount of DPV in control rings from the same lot number.

The number and proportion of participants visits with concentrations indicative of non-adherence (no use) will be compared by study arm using generalized estimating equations (GEE) with a Poisson link, an offset of number of tests for drug concentration, an exchangeable correlation structure and robust errors. The comparisons will be done using the cut-point levels described above for the two study arms: 1) residual ring data for participants assigned to the VR arm and 2) plasma data for participants assigned to Truvada. Trends over time in study product use will be explored using GEE.

This analysis differs slightly from the protocol in two ways. First, since the protocol was written, Dapivirine ring release has become the standard objective measure for vaginal ring use; therefore, Dapivirine plasma levels have been removed from the definition. Second, the outcome will be non-adherence (no use) rather than consistent study product use. No use is the only level of concentration results that are comparable between the two measures of product use. This is also more standard in adherence analyses since the protocol was written.

Reasons for study product non-use will be tabulated by arm. For women randomized to the VR arm, a summary with ring outages collected on the Ring Adherence eCRF will be presented by site. The summary will include ring outage reasons for removals and expulsions, the frequency of outages, and whether any outages were more than 12 hours. For women randomized to Truvada, a summary of missed pills by self-report from the Tablet Adherence eCRF will be presented. The summary will include the number of times per week the participant took the pill (0-7 days), how often the participant skipped the pill (never, rarely, often), and the reasons for not taking the pills.

9.2 Acceptability

For the secondary endpoint of acceptability, all mothers with non-missing responses for the questions (#1 or #2, #5, #6 and #7) on the Behavioral Assessment – Month 3 Follow Up eCRF will be included in the analysis. Mothers with some missing responses to the identified questions will be included in each part of the acceptability analysis as appropriate. The questions are outlined below where <study product> is their randomized assignment:

- Question #1 states “Overall, how much do you like using the pill?” with options 1. Dislike very much, 2. Dislike, 3. Neither like nor dislike, 4. Like, and 5. Like very much. (Reported only for participants assigned to the Truvada arm.)
- Question #2 states “Overall, how much do you like using the vaginal ring?” with options 1. Dislike very much, 2. Dislike, 3. Neither like nor dislike, 4. Like, and 5. Like very much. (Reported only for participants assigned to the DPV VR arm.)
- Question #5 states “Would you be willing to use <study product> for HIV prevention when breastfeeding in the future?” with options 1. Yes, 2. No, and 3. Not sure.
- Question #6 states “If the vaginal ring or oral PrEP were available to you, which product would you prefer to use for HIV prevention?” with options 1. Vaginal ring, 2. Oral PrEP, 3. Either – I find either product acceptable, and 4. Neither – I find neither product acceptable.
- Question #7 states “If the <study product> were available to you, would you prefer to use <study product> or male condoms for HIV prevention?” with options 1. <study product>, 2. Male condoms, 3. Either – I find either product acceptable, and 4. Neither – I find neither product acceptable.

Consistent with the secondary objective to characterize acceptability of study products in breastfeeding women, we will focus on three aspects of acceptability of the study products:

1. Overall attitude about assigned study product.
2. Willingness to use the assigned study product in the future.
3. Preference for:

a. either study product and
b. assigned study product compared to other HIV preventions methods (condoms).

For the first aspect, the participants' overall attitude about the assigned study product will be summarized by study arm. Question #1 ("Overall, how much do you like using the pill") is completed by participants assigned Truvada and Question #2 ("Overall, how much do you like using the vaginal ring") is completed by participants assigned to the VR. A bar chart and a table will be used to summarize the distribution of the responses to these 5-point Likert scale questions. The table will include the number and percentage in each response category and the mean, SD, median, IQR, and range. In addition, the participants who respond with 4 ("Like") or 5 ("Like very much") to Questions #1 and #2 will be considered as having a positive overall attitude about their assigned study product. The binomial proportions along with the corresponding 95% CI for each study arm will be used to assess overall attitude of their assigned study product. The study arms will be compared using the Fisher's exact test. Tables will be presented by arm and by site.

For the second aspect, the number and percentage of participants who would be willing to use their assigned study product in the future will be summarized by study arm. Participants who respond with 1 (yes) to Question #5 (Would you be willing to use <study product> for HIV prevention when breastfeeding in the future?) will be considered as willing to use the study product in the future. The binomial proportions along with the corresponding 95% CI for each study arm will be used to assess the willingness to use the study products in the future. The study arms will be compared using the Fisher's exact test.

For the third aspect part a, the number and percentage of participants who prefer the vaginal ring, oral PrEP, both or neither will be summarized by study arm. The proportions of participants along with the corresponding 95% CI for each option (ring, tablet, both or neither) of Question #6 (If the vaginal ring or oral PrEP were available to you, which product would you prefer to use for HIV prevention) will be used to assess study product preference by study arm. The study arms will be compared using the chi-squared test. For the third aspect part b, we use condom use as the other HIV prevention method. The number and percentage of participants who report the study product to be at least as acceptable as male condom use will be summarized by study arm. Participants who respond with 1 (study product) or 3 (Either – I find either product acceptable) to Question #7 (If the <study product> were available to you, would you prefer to use <study product> or male condoms for HIV prevention?) will be considered as finding the study product at least as acceptable for HIV prevention. The binomial proportions along with the corresponding 95% CI for each study arm will be used to assess the preference of HIV prevention methods. The study arms will be compared using the Fisher's exact test.

10. SAFETY ANALYSES

See sections 8.1 and 8.2 for the description of the analyses of the primary safety endpoints. For the additional safety results below, no formal statistical testing will be performed.

10.1 Adverse Events

The following listings and tables of AEs will be presented separately for mothers and infants:

- A cumulative listing of AEs sorted by arm, site, and participant ID.
- Number and percentages of participants with AEs and number and percentages of AEs by body system/MedDRA preferred term and severity overall by arm, and by site.
- Number and percentages of participants with AEs and number and percentages of AEs by body system/MedDRA preferred term and relationship to study product overall by arm, and by site.
- An AE summary of the total number of participants with AEs and total number of AEs by severity and relationship to study product, overall, for each arm, and each site separately.

10.2 Product Holds

Instances of product hold for mothers as collected on the Product Hold Log eCRF will be described. The number and percentages of mothers who had a product hold at least once, the number of product holds, reasons for product holds, resolution of product hold, and duration of product hold (for those who were instructed to resume product use) will be presented in tables by arm and by site.

10.3 Laboratory Evaluations

Boxplots and tables with summary statistics (see section 5) will be reported for laboratory evaluations performed at the Screening and PUEV Visit, by arm and visit and by site and visit. The following tests will be included: hemogram (hemoglobin, hematocrit, MCV, platelets, WBC, neutrophils, lymphocytes, monocytes, eosinophiles, and basophils) test results as collected on the Hematology eCRF and chemistries (AST, ALT, and creatinine and creatinine clearance) test results as collected on the Chemistry Panel eCRF.

10.4 Pelvic Exam Findings

For mothers, a summary of abnormal pelvic exam findings will include any findings reported on the Pelvic Exam eCRF at any visit after the Enrollment Visit. After Enrollment, pelvic exams are required at PUEV and as indicated for any other visit. The numbers and proportion of participants and participant-visits with any abnormal pelvic exam findings and with specific types of findings will be presented in tables by arm and by site.

10.5 Pregnancy

If any pregnancies are reported for mothers during the study, as collected on the Pregnancy Report eCRF, these will be listed, along with the information from the Pregnancy History and Pregnancy Outcome eCRFs. In addition, pregnancy tests results (positive/negative) as collected on the Pregnancy Test Results eCRF will be summarized by study visit in a table by arm and by site. Pregnancy testing is collected at the PUEV and as indicated at other study follow up visits.

10.6 HIV Testing

For both mothers and infants, if there are any positive Rapid HIV test results, the number and proportion of positive Rapid HIV test results as collected in the HIV Test Results eCRF will be summarized in a table by arm. For any confirmatory results, a listing from the HIV Confirmatory Results and/or Infant HIV Confirmatory Results eCRFs will be presented.

10.7 Sexually Transmitted Infections and Genital Infections

The mothers' STI testing and other vaginal findings results during study follow up as collected on the STI Test Results eCRF will be presented in a listing or tables by arm and by site as appropriate. After the Screening Visit, STIs are collected as clinically indicated. Summaries of testing results will be presented for the following: pH, bacterial vaginosis, wet prep for candidiasis (buds and/or hyphae[yeast]), trichomonas (TV), syphilis screening and confirmatory test, urine NAAT for gonorrhea (GC), chlamydia (c. trachomatis, CT), and trichomonas, and hepatitis B surface antigen.

11. REFERENCES

1. C.J.Clopper and E.S. Pearson, *The use of confidence or fiducial limits illustrated in the case of binomial*, Biometrika, vol. 26, 404-413.

12. CHANGE HISTORY

Identify major changes. Only changes after version 1.0 approval need to be recorded.

Version		Affected Section(s)	Activity Description
Number	**Effective Date**		
2.0	Nov. 4, 2022	Title page, ToC, Sections 1, 2.3, 3.1, 3.2, 5, 6.1, 6.2, 8, 8.3, 9.1, 9.2, 10, 10.1, 10.4, 10.7.	- Added an effective date on title page per SCHARP guidelines - Updated Table of Contents - Clarified that the intent to treat (ITT) approach is not a modified ITT and provided more detail of participants included in the analysis. Changes made in the acronyms section 1.0 and sections 3.1, 3.2, and 8. - Removed the ambiguous text about less informative imputation in section 3.2. - Changed to summaries with the number of non-missing values rather than the number of missing values as described in section 5. - In section 6.1, a reference to section 3.1 was added. - Clarified that infant consent was made by the infant's mother in section 6.3. - To section 8.3, added arithmetic mean, standard deviation, and number of detectable drug levels to drug concentration summaries. The natural log transformations will not be used but figures will be presented using the log scale. Box plots with medians will be used to present drug concentrations over study timepoints. - Provided specific details for secondary adherence endpoint analysis description in section 9.1. Adherence defined as "no use" (rather than "consistent study product use") with identified cut-points for the study products. Dapivirine (DPV) release rate defined for the DPV ring arm. Further details provided for the comparison (GEE) models. Added an explanation of the differences from the protocol. - Clarified that Questions #1 and #2 are study product arm specific. - Removed first sentence of section 10 with clarification provided in section 3.1. - In sections 10.1 and 10.4, clarified that number and percentages of participants with AEs or abnormal pelvic exam findings will be included in addition to the number and percentages of AEs or pelvic exam findings in the summaries. - In section 10.7, text for "Genital Infections" and "other vaginal findings" was added for clarification. - Made grammatical and typographical error corrections (2.3, 3.1, 6.2, 8.3)

From: Barbra Richardson
To: Gundacker, Holly M
Subject: RE: MTN043 SAP version 2.0 approval
Date: Thursday, October 20, 2022 4:06:59 PM

I, Barbra Richardson, Research Professor of Biostatistics, approve. This email is a substitute for wet signature approval for the MTN043 Statistical Analysis Plan, version 2.0 with effective date November 4, 2022.

From: Gundacker, Holly M <hgundack@scharp.org>
Sent: Thursday, October 20, 2022 3:18 PM
To: Barbra Richardson <barbrar@uw.edu>
Subject: MTN043 SAP version 2.0 approval

Hi Barb,

Attached is the MTN043 Statistical Analysis Plan version 2.0 for your approval. If you approve this document please reply to this email with the following statement:

"I, [name and title], approve. This email is a substitute for wet signature approval for the MTN043 Statistical Analysis Plan, version 2.0 with effective date November 4, 2022."

Thanks!
Holly

Holly Gundacker (she/her)
SCHARP Statistical Research Associate
Statistical Center for HIV/ADIS Research and Prevention (SCHARP)
Vaccine and Infectious Disease Division (VIDD)
Fred Hutchinson Cancer Center
O 206.667.6480
hgundack@scharp.org

From: Gundacker, Holly M
To: Gundacker, Holly M
Subject: RE: MTN043 SAP version 2.0 approval
Date: Thursday, October 20, 2022 4:54:30 PM

I, Holly Gundacker, Statistical Research Associate, approve. This email is a substitute for wet signature approval for the MTN043 Statistical Analysis Plan, version 2.0 with effective date November 4, 2022.

From: Gundacker, Holly M <hgundack@scharp.org>
Sent: Thursday, October 20, 2022 3:19 PM
To: Gundacker, Holly M <hgundack@scharp.org>
Subject: MTN043 SAP version 2.0 approval

Holly,

Attached is the MTN043 Statistical Analysis Plan version 2.0 for your approval. If you approve this document please reply to this email with the following statement:

"I, [name and title], approve. This email is a substitute for wet signature approval for the MTN043 Statistical Analysis Plan, version 2.0 with effective date November 4, 2022."

Thanks,
Holly

Holly Gundacker (she/her)
SCHARP Statistical Research Associate
Statistical Center for HIV/ADIS Research and Prevention (SCHARP)
Vaccine and Infectious Disease Division (VIDD)
Fred Hutchinson Cancer Center
O 206.667.6480
hgundack@scharp.org