STATISTICAL ANALYSIS PLAN

MTN-035

Acceptability, Tolerability, and Adherence of Three Rectal Microbicide Placebo Formulations among HIV Seronegative Cisgender Men, Transgender Men and Transgender Women Who Engage in Receptive Anal Intercourse

Statistical Center for HIV/AIDS Research and Prevention

Elizabeth Brown, ScD

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Date: Monday, January 25, 2021 2:51:17 PM

I, Elizabeth Brown, Professor, approve MTN-035_Statistical_Analysis_Plan_V1.0_01252021 document. This email is a substitute for a handwritten signature.

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Thank you,
Yuqing

From: Jiao, Yuqing
To: Jiao, Yuqing
Subject: RE: Please review and approve MTN-035_Statistical_Analysis_Plan_V1.0_01252021
Date: Monday, January 25, 2021 3:04:08 PM

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Thank you,
Yuqing

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1. LIST OF ABBREVIATIONS AND ACRONYMS

Term/Abbreviation	Definition
AE	adverse event
CASI	computer assisted self-interview
CI	Confidence interval
CRF	case report from
PUEV	product use end visit
RAI	receptive anal intercourse

2. INTRODUCTION

This SAP is intended to describe the final primary and secondary analyses upon completion of MTN-035.

2.1 General Design Considerations

Short Title: Rectal Microbicide Acceptability, Tolerability, and Adherence

Funders: Division of AIDS, NIAID, NIMH, NICHD, US NIH

Protocol Chair: José A. Bauermeister, PhD, MPH

Sample Size: MTN-035 will enroll approximately 210 participants

Study Population: HIV-uninfected cisgender men, transgender men (TGM) transgender women (TGW) aged 18-35 years who engage in receptive anal intercourse (RAI)

Study Sites: Sites selected by MTN Executive Committee

Study Design: Multi-site, randomized-sequence, three-period, open label crossover study

Study Duration: Approximately 3.5 months of follow-up with a projected accrual period of 9-12 months

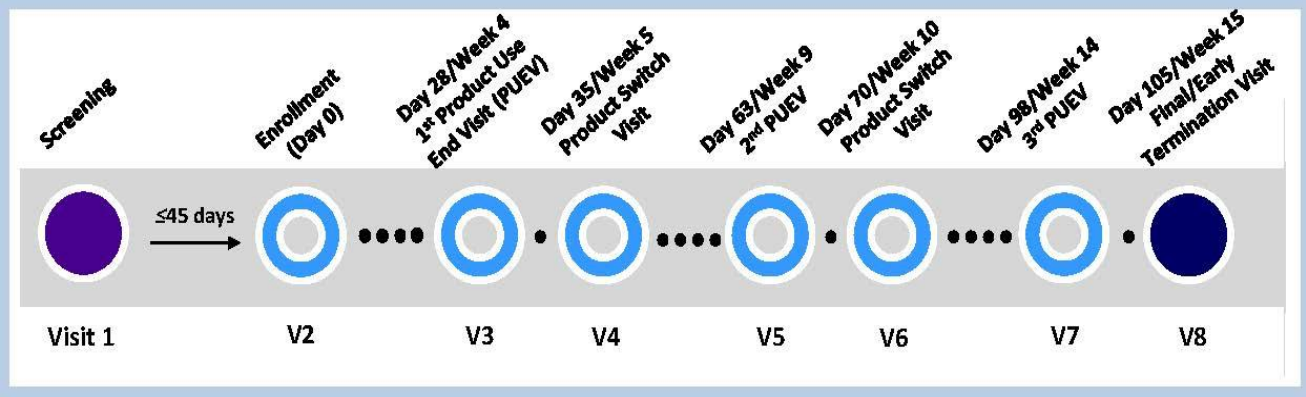
Study Products: Placebo rectal insert
Placebo rectal douche
Placebo rectal suppository

Study Regimen: Participants will be randomized (1:1:1:1:1:1) to study product sequences A-F (see Table 1 below). At the start of each 4-week product use period, they will receive either rectal inserts, rectal douches, or rectal suppositories and be instructed to use their assigned study product prior to each RAI encounter during that period. Participants who do not have RAI in a given week will be asked to use the product without sex. There will be a 1-week washout period between each of the three product use periods.

Table 1: MTN-035 Study Product Regimen

Sequence	N	Period 1 (4 weeks)	Washout period (~1 week)	Period 2 (4 weeks)	Washout period (~1 week)	Period 3 (4 weeks)
A	35	Rectal insert	--	Rectal douche	--	Rectal suppository
B	35	Rectal douche	--	Rectal suppository	--	Rectal insert
C	35	Rectal suppository	--	Rectal insert	--	Rectal douche
D	35	Rectal insert	--	Rectal suppository	--	Rectal douche
E	35	Rectal douche	--	Rectal insert	--	Rectal suppository
F	35	Rectal suppository	--	Rectal douche	--	Rectal insert

Figure 1: MTN-035 Study Visit Schedule



As displayed in Figure 1, participants will receive and start the use of the first product, according to the randomized sequence, at enrollment visit and will end the use of that first product at week 4 (visit 3). After a 1-week washout period, the participant will receive and start use of the second product, according to the randomized sequence, at visit 4 and end the use of that second product at week 9 (visit 5). Similarly, the participant will receive and start the third product use after a 1-week washout period following the second product use end visit. The final termination visit (visit 8) will occur after the third product use end visit (visit 7).

2.2 Study Objectives and Endpoints

Primary Objectives:

Acceptability and Tolerability

- To evaluate the acceptability and tolerability of each study product when applied rectally and used prior to RAI
  - Placebo rectal insert
  - Placebo rectal douche
  - Placebo rectal suppository

Adherence

- To evaluate adherence to each study product prior to RAI over a 4-week-long period
    - Placebo rectal insert
    - Placebo rectal douche
    - Placebo rectal suppository

Exploratory Objective:

Relative Acceptability and Tolerability

- To evaluate the relative acceptability and tolerability between study products when applied rectally and used prior to RAI
    - Placebo rectal insert
    - Placebo rectal douche
    - Placebo rectal suppository

Primary Endpoints:

Acceptability and Tolerability

- For each study product, participant self-report of likelihood of product use if shown to be effective

Adherence

- Per participant report, percentage of occasions when each study product was used as instructed (per protocol)

Safety

- Grade 2 or higher related adverse events (AEs) as defined by the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1, July 2017, and/or Addenda 1, 2 and 3 (Female Genital [Dated November 2007], Male Genital [Dated November 2007] and Rectal [Clarification Dated May 2012] Grading Tables for Use in Microbicide Studies)

Exploratory Endpoint:

Relative Acceptability and Tolerability

- Conjoint analysis of participant acceptability and tolerability between the three study products

2.3 Randomization

Participants will be randomly assigned with the ratio 1:1:1:1:1:1 to one of six study product application sequences. The randomized assignments will be in blocks to keep the balance of equal allocation.

2.4 Blinding

Participants and site staff will be unblinded throughout the trial due to this study being an open label trial.

2.5 Sample Size and Power

There is no control group for comparison in this study. The goal is not to compare each product to some standard but instead to estimate overall rates of acceptability, adherence and safety.

Primary Endpoints – Acceptability/Tolerability and Adherence

Based on previous studies we expect to observe a high rate of acceptability (>80%) which equates to ≤20% of participants reporting a low likelihood of using the product in the future (≤3 on the rating scale). Likewise, we expect to observe a high rate of participants reporting taking at least 90% of expected doses.

The table below shows the exact confidence intervals for acceptability and adherence, assuming α=0.05 based on the Binomial distribution and varying rates of acceptability and adherence. The power to rule out <50% and <70% acceptability or adherence given the rates is also shown. Calculations assume the outcomes are observed in 200 of the participants (5% loss to follow-up). For example, if the true acceptability or adherence rate is 90%, we have 100% power to rule out a true acceptability or adherence rate of 75% or lower. If we observe an 80% acceptability or adherence rate, the corresponding 95% confidence interval (CI) will be (74%, 85%).

Table 2: Exact 2-sided 95% Confidence Intervals Based on Various Rates of Acceptability or Adherence Endpoints

Acceptability rate / adherence rate	95% CI if rate is observed	Power to rule out 50% if rate is true	Power to rule out 70% if rate is true	Power to rule out 75% if rate is true
95%	(91, 98)	100%	100%	100%
90%	(85, 94)	100%	100%	100%
85%	(79, 90)	100%	100%	93%
80%	(74, 85)	100%	90%	35%

Primary Endpoint – Safety

To characterize the statistical properties of the safety endpoint, the table below presents the probability of observing zero, at least one, and two or more safety endpoints among 200 participants (allowing for the possibility of 5% dropout) assuming various "true" event rates. By assuming that we may only have safety evaluations on 200 participants, these calculations are conservative. However, safety analyses will include all enrolled participants.

Table 3: Analysis of Safety Event Frequency

Event Rate	P(0 events | n=200)	P(≥1 event | n=200)	P(≥5 events | n=200)	P(≥10 events | n=200)
0.1%	82%	18%	0%	0%
0.5%	37%	63%	0%	0%
1%	13%	87%	5%	0%
2%	2%	98%	37%	1%
5%	0%	100%	97%	55%

3. GENERAL DATA ANALYSIS CONSIDERATIONS

3.1 Analysis Set(s)

Safety endpoints will be assessed on a modified intent-to-treat analysis set, which will include all participants enrolled in the study who received at least one of the study products during the study. Primary acceptability, tolerability and adherence endpoints will be assessed on a per-protocol (PP) analysis subset, which will consist of participants who received all three study products and completed the corresponding product use end visits (PUEVs).

3.2 Statistical Analysis Issues

A retention rate of 95% is targeted. Based on previous MTN trials, minimal missing data is expected. If missing data rates are higher than anticipated (over 10%), sensitivity analyses will be conducted to assess the impact of missing data on trial inference. If missing data rates are over 10 and assuming missing data are ignorable, we will use multiple imputation based on all available baseline predictors and available trial outcomes. Otherwise, we will complete analyses using available data.

For primary safety analysis, safety endpoints will be classified by the product used at that particular period, starting from date of enrollment or most recent product switch visit. If a participant did not receive a particular product then that participant-period will be excluded from the analysis. Due to COVID-19, some participants have received multiple products in advance or have received product through mail. The actual product switch visit date for the corresponding product will be used as the start of the product use period instead of the date participants received the product. A safety endpoint will be considered to have occurred during a specific product use period if the onset date of the AE is on or after start of the product use period (enrollment or switch visit date) and before the start of the next product use period (switch visit date or end of study).

4. INTERIM ANALYSIS AND DATA MONITORING COMMITTEE

No interim statistical analysis was planned or performed for the MTN-035 study.

Two study monitoring committee (SMC) review were conducted for MTN-035 on October 10, 2019 and May 4, 2020.

5. GENERAL ANALYSIS METHODS

When use of descriptive statistics is required for the assessment or comparison of study product group characteristics, the following statistics will be reported: for categorical variables, the number and percent in each category; for continuous variables, the mean, median, standard deviation, quartiles, and range (minimum, maximum).

6. TRIAL PARTICIPANT DISPOSITION

6.1 Disposition of Participants

6.1.1 Screening and Enrollment

Dates of site activation, first enrollment, and last enrollment, as well as the number or participants screened, number and percentage of participants enrolled, and the screening-to-enrollment ratio will be displayed in a table overall and by site.

6.1.2 Retention

The number and percentage of participants who are expected, retained, missing, and lost-to-follow-up or terminated with respect to the visit will be displayed for each visit in a table overall and by site, and in a table overall and by randomized sequence.

Interim visits completed to make up for a missed visit will be included in both tables. If a missed visit is made up by completing an interim visit, then the missed visit will be considered as completed in the retention tables.

6.1.3 Treatment Discontinuation

The number and percentages of participants who ended product use early, as well as the reason for terminating product use early, will be presented in a table overall and by site and in a table overall and by randomized sequence.

6.1.4 Study Discontinuation

The number and percentages of participants who completed the study, as well as the reasons for non-completion, will be displayed in a table overall and by site.

6.1.5 Completion of Procedures

The completion of required and expected procedures will be displayed in a table overall and by site. The table will display the number and percentages of participant-visits for each procedure.

6.2 Treatment Exposure

The number and percentage of participants who received each of the three study products, along with the number and percentage of participants who completed the corresponding product use end visit (PUEV) will be displayed in a table overall and by site, and in a table overall and by randomized sequence. In addition, the number and percentage of participants who received all three study products and completed all three PUEV will also be displayed.

6.3 Protocol Deviations

Protocol deviations occurring in MTN-035 will be summarized in a table for each type of deviation, overall and by site, and in a listing of deviation events.

7. BASELINE DATA

Baseline demographic characteristics of the participants, such as age, age group, sex at birth, gender, ethnicity (U.S sites), race (U.S sites), ethnic group or tribe (international sites) will be summarized and displayed in a table overall and by site, and in a table overall and by randomized sequence. Descriptive statistics will be presented for age. Frequency counts and percentages will be presented for age group, sex at birth, gender, ethnicity (U.S sites), race (U.S sites), ethnic group or tribe (international sites). No formal comparisons are planned.

Other individual characteristics of the participants at baseline, including baseline medical history, baseline rectal exam findings, and baseline STI findings, will be presented in listings if applicable.

8. PRIMARY ENDPOINTS

The acceptability, tolerability and adherence primary endpoints will be evaluated on the per-protocol subset of participants, i.e. participants who received all three study products and completed the corresponding PUEV, and thus provide relevant data from all three periods of study. Assessment of these endpoints will be based on responses from the participants to the computer assisted self-interviews (CASI) at the three PUEVs (Visits 3, 5 and 7). To aid in the interpretation of the resulting analyses, baseline characteristics of the per-protocol subset of participants including, but not limited to, study site, sequence, sex/gender, and age will be described and compared to those of participants who were lost to follow-up.

Acceptability and Tolerability
The acceptability and tolerability endpoint will be based on question H1 of the CASI:

> "Think about the positive and negative experiences you have had using the rectal Insert during the past 4 week period. If this rectal insert was available and it provided some protection against HIV, how likely would you be to use it before receptive anal sex?".

Responses to this question for each product were captured from visual Likert scale from 1 (very unlikely) to 10 (very likely). Participants' responses corresponding to the three product use periods will be displayed in tables by study product, by study product and site and by study product and randomization sequence.

To compare acceptability and tolerability between study products, the endpoint will be operationalized as binary, with scores 1 to 6 grouped as "low acceptability" and scores 7 – 10 as "high acceptability". Number and percentage of participants giving a "high acceptability" score will be display in tables by study product, with exact binomial 95% confidence intervals (Clopper-Pearson method).

Adherence

Two endpoints related to adherence will be assessed: adherence to use per-protocol and adherence to use per-anal-sex-act. These are defined as follows:

1. **Adherence to use per-protocol:** Number and proportion of participants who reported not having missed a rectal [enema/douche, insert or suppository] application in the previous 4 weeks, according to the participants' response to question B0 of the CASI:

   > B0. The following questions refer to your use of the study provided rectal ***[study product: douche/enema, insert or suppository]*** over the past 4 weeks. You were asked to insert the study provided rectal ***[study product]*** in your rectum at least once a week during the past 4 weeks. However, for different reasons, people might have encountered difficulties using the ***[study product]***. Thinking about your experience during these past four weeks, in how many of the weeks did you miss a rectal ***[study product]*** application?
   >
   > 0 weeks
   > 1 week
   > 2 weeks
   > 3 weeks
   > 4 weeks

   Responses to question B0 will be tabulated by study product, study product and sequence and study product and site, along with the number and proportion of participants fully adherent (those

who reported 0 weeks missing application), with 95%confidence intervals (Clopper- Pearson). If needed, additional categories of invalid/missing responses will be included.

2. **Adherence per sex-act:** Proportion of times when participants reported having used the study-provided rectal douche/enema, insert or suppository before receptive anal sex. For participants who reported at least 1 anal sex act in the previous 4 weeks, this proportion will be obtained by dividing the number reported in Question B2 over the number reported in Question B1 of the CASI:

   B1. How many times did a partner put a penis in your rectum during the past 4 weeks?

   B2. Of those [ ] times, how many times did you use the study-provided rectal ***[study product: douche/enema, insert or suppository]*** BEFORE receptive anal sex? (If you did not use the douche prior to anal sex, please enter '0'.)

   Responses to Questions B1 and B2 will be cross tabulated, by study product and by study product and site. If needed, additional categories of invalid/missing responses will be included. Summary statistics (mean and SD, median and IQR) of participants' per sex-act adherence, as defined above, will be displayed by study product and by study product and site.

9. PRIMARY SAFETY ANALYSES

All participants enrolled and administered a study product will be assessed for safety.

9.1 Primary Safety Analyses

The primary safety endpoint is defined as follows:

> Grade 2 or higher related adverse events (AEs) as defined by the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1, July 2017, and/or Addenda 1, 2 and 3 (Female Genital [Dated November 2007], Male Genital [Dated November 2007] and Rectal [Clarification Dated May 2012] Grading Tables for Use in Microbicide Studies)

The number and the percentage of participants experiencing each safety endpoint will be tabulated by study product. This table will display a summary for any primary safety endpoint and for each individual safety endpoint. Each participant will contribute once in each category (i.e., only for highest severity AE for each participant) for the calculation of event rates for each study product. Exact binomial confidence intervals (Clopper-Pearson) will be calculated for each safety endpoint, even if no events occur for an endpoint for a particular study product.

9.2 Adverse Events

Summaries of adverse events will include a cumulative listing of adverse events, a table displaying the total number of adverse events by severity grade and relationship to study product, a table of the incidence of adverse events by MedDRA organ system class/preferred term and severity, and a table of incidence of adverse events by MedDRA organ system class/preferred term and relationship to study product. Tables above will be displayed by site and displayed by study product. The listing will include the study product used period when the AE occurred.

9.3 Other Safety Measures

Additional safety summaries include:

The number and percentage of participants with a positive result for hCG for pregnancy and HIV will be displayed in a table overall and by site if applicable. A listing of participants with positive results of STI testing (syphilis, trichomonas, gonorrhea, chlamydia) after first study product use will be presented if applicable.

A listing of participants with abnormal findings on rectal exam after first study product used will be presented.

A listing of all concomitant medications will be presented.

10. Exploratory Analysis

In addition to the analyses described above, the following analyses will be conducted to compare and estimate the relative acceptability/tolerance, adherence and safety between the three study products. These analyses will also allow to assess potential effects of the order of product use on each of the endpoints.

A logistic mixed effects regression model will be used to estimate the relative odds (odds ratios) of high acceptability between the different study products. A similar logistic mixed effects regression model will be used to estimate the relative odds (odds ratio) of full per-protocol and per-sex-act adherence between the different study products. To account for the cross-over design of the study, a random intercept at the participant level will be included in the models. Estimates of the odds-ratios, along with 95% confidence intervals and associated p-values will be reported. If feasible, testing for effects of the order of product use will be done by including interaction terms between study product and randomized sequence in the model.

A logistic mixed effects regression model will be used to estimate the relative odds (odds ratio) of having a primary safety endpoint, as defined in section 9.1, between the different study products. A Poisson mixed effects regression model will be used to compare the incidence (expected counts) of AEs per study period between the study products. If feasible, interaction terms between study product and randomized sequence will be included in the model to test the effect of order of product use on the safety endpoints.

11. References

Acceptability, Tolerability, and Adherence of Three Rectal Microbicide Placebo Formulations among HIV Seronegative Cisgender Men, Transgender Men and Transgender Women Who Engage in Receptive Anal Intercourse (MTN-035). Microbicide Trials Network (MTN) clinical study protocol, Version 1.0, June 15, 2018

Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1, July 2017

Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Publish Date: December 2004, Addendum 1, Female Genital Grading Table for Use in Microbicide Studies, Version 1.0, November 2007.

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Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Addendum 3, Rectal Grading Table for Use in Microbicide Studies, Clarification dated May 2012.