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Statistical Center for HIV/AIDS Research and Prevention

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STATISTICAL ANALYSIS PLAN

MTN-026

A Randomized, Double Blind, Placebo-Controlled, Phase 1 Safety and Pharmacokinetic Study of Dapivirine Gel (0.05%) Administered Rectally to HIV-1 Seronegative Adults

Statistical Center for HIV/AIDS Research and Prevention

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Approval Signature Page

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I have read this Statistical Analysis Plan and approve its contents.

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1. LIST OF ABBREVIATIONS AND ACRONYMS

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
CV	coefficient of variation
DPV	dapivirine
eCRF	electronic case report form
HIV	Human Immunodeficiency Virus
HIV-1	Human Immunodeficiency Virus type 1
PD	pharmacodynamics
PK	pharmacokinetics
SAP	statistical analysis plan
SGOT	serum glutamic-oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SMC	study monitoring committee
STI	sexually transmitted infection
$t_{1/2}$	terminal elimination half-life

2. INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to provide a description of the statistical analyses that will be conducted to assess the safety, pharmacokinetics and acceptability of the dapivirine gel administered into the rectum, when compared to a placebo gel, among HIV-uninfected men and women.

2.1 General Design Considerations

The following is a protocol summary of the study.

Protocol title:	A Randomized, Double Blind, Placebo-Controlled Phase 1 Safety and Pharmacokinetic Study of the Dapivirine Gel (0.05%) Administered Rectally to HIV-1 Seronegative Adults
Short Title:	Dapivirine Gel Rectal Safety and PK Study
Clinical Phase:	Phase 1
IND Sponsor:	DAIDS
Protocol Chair:	Ross D. Cranston, MD, FRCP
Sample Size:	approximately 27 participants
Study Population:	HIV-uninfected men and women between the ages of 18 and 45 years (inclusive)
Study Sites:	Sites selected by the MTN leadership
Study Design:	Phase 1, two-arm, placebo-controlled, double-blinded, multi-site, randomized trial (2:1)
Study Duration:	Approximately 40 days of follow-up per participant is planned with a projected accrual period of 6-8 months
Study Products:	Dapivirine gel (0.05%)

Universal HEC placebo gel

Study Regimen: Participants will be randomized in a 2:1 ratio to receive a single dose of either dapivirine gel or universal HEC placebo gel rectally, followed by 7 daily doses of the same product to be administered under direct observation in the clinic.

2.2 Study Objectives and Endpoints

Primary Objectives:

Safety

- To assess the safety of dapivirine gel formulation when applied rectally.

Pharmacokinetics

- To characterize the systemic and compartmental pharmacokinetics of dapivirine gel following rectal application.

Secondary Objectives:

Acceptability

- To identify product attributes considered likely to challenge and facilitate future sustained use of rectally applied dapivirine gel.

Mucosal Safety

- To evaluate the mucosal safety of dapivirine gel when applied rectally.

Exploratory Objectives:

Ex Vivo Efficacy

- To assess the preliminary (ex vivo) efficacy of dapivirine gel formulation after product is applied rectally.

Pharmacokinetics

- To characterize the vaginal pharmacokinetics of dapivirine gel following rectal application in women.

Primary Endpoints:

Safety

- The proportion of participants with:
    - Grade 2 or higher 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).

Pharmacokinetics

- Dapivirine concentrations
    - Blood
    - Rectal fluid
    - Rectal mucosal tissue homogenates.

Secondary Endpoints:

Acceptability

- Product attributes considered likely challenge future sustained use

Mucosal Safety

- Rectal Proteomics
- Rectal transcriptome
- Rectal microflora
- Rectal histology
- Rectal tissue flow cytometry

Exploratory Endpoints:

Ex Vivo Efficacy

- Changes in HIV-1 p24 levels in colorectal explant culture supernatant
- Anti-HIV activity in rectal fluid
- Anti-HIV activity in cervicovaginal fluid (in women)

Pharmacokinetics

- Dapivirine concentrations
    - Cervicovaginal fluid (in women)
    - Cervical tissue (in women)

2.3.1 Randomization

Participants were randomized in a 2:1 ratio to the dapivirine and placebo arms, stratified by study site.

Participants were also sequentially assigned in a 1:1 ratio to provide intensive PK samples at either 30-60 or 120 minutes after administration of the gel on Visits 3 and 13. Participants were also sequentially assigned in a 1:1:1 ratio to provide intensive PK samples at either Visits 4 and 14, Visits 5 and 15 or Visits 6 and 16.

The randomization scheme has been generated and maintained by the MTN SDMC.

2.3.2 Blinding

Study staff and participants are blinded to the random treatment assignment of all study participants.

2.3.3 Sample Size and Power

The proposed sample size for this study was 27 participants, with 18 in the active arm and 9 in the control arm. The study has power to detect only very large differences in safety event rates between active and control arms. With 5% significance level, the study has 85% power to detect a rate in safety endpoint greater than 67% in the active arm when assuming an 11.2% rate in the control arm. On the other hand, the proposed sample size ensures a reasonable frequency of safety events that are not too rare. For example, if the true event rate of an adverse event is 10%, then the probability of observing at least one adverse event in the study is 85%.

3. GENERAL DATA ANALYSIS CONSIDERATIONS

3.1 Analysis Set(s)

The analysis set for the primary safety endpoint will include all enrolled participants who received at least one dose of the study product (either the dapivirine or the placebo gel). The analysis set for the primary PK endpoint will include enrolled participants who were assigned to receive the active treatment and who received at least one dose of the study product (dapivirine gel).

Participants enrolled but lost to follow-up between enrollment visit (Visit 2) and the first dosing visit (Visit 3) or who were never administered the gel will not be included in the analysis sets. Enrolled participants who received the study product but were found to be ineligible (enrollment violations) will preferably be included in the analysis sets, in order to capture all adverse events potentially related to the study product. Sensitivity analysis of the primary safety endpoint excluding these participants will be conducted.

All the participants to be excluded from the analysis sets will be identified prior to data lock and to the unblinding of the data and the reasons for exclusion will be properly documented.

3.2 Statistical Analysis Issues

Based on previous MTN trials, minimal missing data is expected. Any missing data will be tabulated as a separate category when reporting data summaries.

If missing data rates are higher than anticipated (over 10%), sensitivity analyses for the primary safety endpoint will be conducted. For these sensitivity analyses, we will impute extreme values to the missing data and report the resulting analyses.

4. INTERIM ANALYSIS AND DATA MONITORING COMMITTEE

No interim statistical analysis was planned or performed for the MTN-026 study.

One safety monitoring committee (SMC) review was conducted for MTN-026, on May 14, 2018, for which a closed report was produced. Evaluation of safety was based on descriptive tables of adverse events, with no formal statistical testing.

5. GENERAL ANALYSIS METHODS

Descriptive statistics will be used to summarize continuous (mean and standard deviation, median and interquartile range, quartiles, range, and number of missing data values) and categorical (frequencies, relative frequencies, percentages, and number of missing data values) outcomes. Line, scatter, and box plots will be used, as appropriate, to visualize trends in longitudinally repeated measures.

Confidence intervals will be obtained using the Clopper-Pearson method for binary outcomes, while for continuous outcomes these will be based on the Student's-*t* statistic. Unless otherwise specified, 95% confidence intervals will be provided.

When use of formal testing to assess differences between participants in active and placebo arms are required, the following methods will be used: for binomial response variables, a Fisher's exact test; for continuous variables, t-tests or nonparametric methods. Unless otherwise specified, results from two-sided hypothesis tests will be reported.

6. TRIAL PARTICIPANT DISPOSITION

6.1 Disposition of Participants

Enrollment of participants took place at three study sites: Birmingham, AL, and Pittsburgh, PA, in the US and Bangkok in Thailand.

6.1.1 Study Screening and Enrollment

The number of participants screened, the number of participants who were enrolled and not enrolled, the number of participants with each reason for not enrolling, and the number of participants considered evaluable for each primary and secondary endpoint will be displayed in tables overall and by site. These summaries will also be displayed by arm in a CONSORT diagram.

Dates of site activation, first enrollment and last enrollment, as well as the number of participants enrolled the screening-to-enrollment ratio will be displayed in tables overall and by site and by arm.

6.1.2 Retention

For each visit, a participant is expected to have returned to the site clinic within a specified visit window, based on the participant's date of enrollment visit or, for sampling visits, based on the previous dosing visit. The proportion of participants 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, missed visits, and lost-to-follow-up or terminated participants, overall and by arm and by site. No formal statistical testing will be conducted.

6.1.3 Treatment Discontinuation

The number and percentage of participants who ended product use early (prior to visit 13), as well as the reasons for terminating product use early, as collected on the Treatment Discontinuation electronic Case Report Form (eCRF), will be presented in tables by arm and by site. Reasons for ending study product use early include the following: acquisition of HIV infection, Adverse Event, reported use of prohibited medications, pregnancy, breastfeeding, anorectal STIs, participant unable/unwilling to comply with required study procedures or otherwise might be put at undue risk to their safety and well-being by

continuing product use according to judgment of IOR/designee, other. No formal statistical testing will be conducted.

6.1.4 Study Discontinuation

The number and percentage of the participants who completed the study, as well as the reasons for non-completion, as collected on the Study Discontinuation electronic Case Report Form (eCRF), will be presented in tables by arm and by site. Reasons for non-completion include the following: death, withdrawal of consent by participant, lost to follow-up, investigator decision, study terminated by sponsor, pregnancy, HIV infection, permanent study product discontinuation, other. No formal statistical testing will be conducted.

6.1.5 Visit Adherence: Completion of Procedures

Visit adherence will be summarized by completion of required and expected procedures overall and by site. This will be done for the following procedures (we mark with asterisk (*) those procedures that are expected only for female participants): 1) Physical exam; 2) vital signs; 3) sexual lubricant form; 4) pregnancy tests *; 5) chemistries; 6) pelvic exam *; 7) rectal exam; 8) plasma archive; 9) plasma for PK; 10) HIV Test; 11) CVL for PD*; 12) cervicovaginal fluid for PK *; 13) cervical tissue for PK *; 14) rectal fluid for PK; 15) rectal tissue for PK; 16) rectal fluid for mucosal safety; 17) rectal tissue for mucosal safety; 18) rectal tissue for PD; 19) rectal enema effluent for PK/PD; 20) behavioral assessment; 21) In-depth interview. Tables displaying the number and percentages of participant-visits for each of these categories by arm and site will be presented. No formal statistical testing will be conducted.

6.2 Treatment Exposure

Exposure to treatment, as collected on the Directly Observed Dosing Log eCRF at visit 3 and visits 7 to 13 will be described. The number and percentage of participants for whom the gel application was (i) observed in clinic, (ii) done in home, not observed or (iii) not done, will be displayed in tables by visit and site and by visit and study arm.

6.3 Protocol Deviations

A table with the number and type of protocol deviations will be presented, by site and by arm. A listing of all the protocol deviations will be presented, including the description of the deviations.

7. BASELINE DATA

7.1 Demographics

Demographics and baseline characteristics of participants as collected on the Demographics eCRF will be presented by arm and by site. These characteristics include age (continuous in years, and in categories of five years), sex at birth, gender identity, marital and cohabitation status, highest level of education, race, ethnicity, and income source. No formal statistical testing will be performed.

7.2 Baseline Medical History

A listing of all baseline medical history events as collected on the Baseline Medical History Log eCRF will be presented, including grade, severity/intensity, whether the condition is ongoing, and the beginning and end dates of the event. No formal statistical testing will be performed.

7.3 Vital Signs

Baseline vital signs as collected on the Vital Signs eCRF at Screening and Enrollment will be presented by arm and visit and by site and visit. Summary statistics appropriate for the measurement scale will be used to describe distribution of height (at Screening only), weight, body temperature, systolic blood pressure, diastolic blood pressure, pulse, and rate of respiration. No formal statistical testing will be performed.

7.4 Physical Exam

Baseline physical exam results as collected on the Physical Exam eCRF at Screening and Enrollment will be presented in tables by arm and visit and by site and visit. Number and percentage of participants experiencing any abnormal physical findings at each visit will be presented as well as the number and proportions of participants with findings for the following organ systems and body parts: general appearance; head, eye, ear, nose, and throat; oral mucosa; neck; lymph nodes; heart/cardiovascular; lung/respiratory; abdomen; extremities; neurological; skin; and other system. No formal statistical testing will be performed.

7.5 Anorectal and Pelvic Exams

Baseline results from the anorectal exam (all participants) and the pelvic exam (female participants only), as collected on the Anorectal Exam and Pelvic Exam eCRFs at Screening and Enrollment will be presented in tables by arm and visit and by site and visit. Number and percentage of participants experiencing any abnormal findings at each visit will be presented as well as the number and proportions of participants with specific types of anorectal or pelvic exam findings. No formal statistical testing will be performed.

7.6 Hematology

Baseline hemogram and differential results as collected on the Hematology eCRF at Screening will be presented in tables by arm and by site. Summary statistics appropriate for continuous variables will be used to describe each of the following: hemoglobin, hematocrit, MCV, platelets, WBC, neutrophils, lymphocytes, monocytes, eosinophils, and basophils. No formal statistical testing will be performed.

7.7 Laboratory Evaluations

Baseline laboratory results as collected on the Local Laboratory eCRF at Screening and Enrollment will be presented in tables by arm and visit and by site and visit. Summary statistics appropriate for continuous variables will be used to describe each of the following: AST (SGOT), ALT (SGPT), and creatinine values. No formal statistical testing will be performed.

8. PRIMARY ENDPOINT ANALYSES

8.1 Adverse Events

For the analysis of the primary safety endpoint, we will present the number and the proportion of participants experiencing at least one Grade 2 or higher adverse event, overall and by study arm. Each participant will contribute once in each category (i.e., only the highest severity AE of each participant) for the calculation of event risks. Exact binomial confidence intervals, using the Pearson-Clopper method, will be provided and Fisher's Exact test will be used to test for differences in risks between the arms.

Additionally, we will also present the following listings and tables of Adverse Events. No formal statistical testing will be conducted.

- The total number (and percentage) of adverse experiences by severity and relationship to study product, overall and for each study arm and for each site.

- Number and percentages of adverse experiences by body system/MedDRA preferred term and severity, by study arm and by site.

- Number and percentages of adverse experiences by body system/MedDRA preferred term and relationship to study product, by study arm and by site.

- Cumulative listing of Adverse Events, with arm, site and participant ID, MedDRA preferred term and AE verbatim, severity, relationship to study product, date reported to site, onset date, outcome date, duration in days, treatment, and outcome.

8.2 Pharmacokinetics Evaluations

8.2.1 Dapivirine concentrations in blood, rectal fluid and rectal tissue

For participants in the active arm only, we will report the dapivirine concentration in blood at all time points where a sample was obtained. At each sampling time point, the concentration of dapivirine will be summarized using descriptive statistics (mean, standard deviation, median, interquartile range and range, along with number of participants). Also, 95% confidence intervals for the mean concentration will be obtained, using a Student's-t distribution approximation. Given the small number of samples that are expected for some sampling points, a log transformation of the concentrations will be considered if the data suggest strong skewness. No formal statistical testing will be performed.

For concentrations of dapivirine that fall below the corresponding assay's lower limit of quantification (LLOQ), we will use a value equivalent to half the LLOQ of the assay. The number and percentage of samples that fall below the LLOQ for any given sampling time point will be reported.

The time-points in the proposed sampling schedule were selected to roughly provide a description of some important PK characteristics. The sample taken 30-60 min after dosing time will be used to describe the concentrations soon after a gel dose. The sample taken 2 h after dosing will be used to roughly approximate peak concentrations. Concentrations from 2 h, 24h, 48 h and 72 h after dosing will be used to estimate terminal elimination half-life. The sample taken at dosing time after multiple doses will be used to estimate the concentration accumulation with multiple dosing (approach to steady state).

The following figures and tables are planned to describe the concentration of dapivirine in blood, rectal fluid and rectal tissue:

<u>Dapivirine concentrations in blood</u>

- Table summarizing the concentration of dapivirine after single dose: at time of dosing (visit 3), 30-60 min (visit 3), 120 min (visit 3), 24 h (visit 4), 48 h (visit 5) and 72 h (visit 6) after the time of dosing.
- Table summarizing the concentration of dapivirine after the first of multiple doses: at time of dosing (visit 7) and 24 h after time of dosing (visit 8).
- Table summarizing the concentration of dapivirine after multiple doses: at time of dosing (visit 13), 30 – 60 min (visit 13), 120 min (visit 13), 24 h (visit 14), 48 h (visit 15) and 72 h (visit 16) after time of dosing.

- Individual concentration profiles for each participant (dapivirine concentration in blood over time), after single dosing (Visits 3 to 6) and multiple dosing (Visits 13 to 16) overlaid in one plot per participant.
- Individual concentration profiles after single dosing (Visits 3 to 6) for all participants, overlaid on a single plot.
- Individual concentration profiles after multiple dosing (Visits 13 to 16) for all participants, overlaid on a single plot.
- Plot of the median of dapivirine concentration in blood over time, with vertical bars indicating 95% confidence interval. Separate plots for concentrations after single and after multiple dosing, as well as overlaid on a single plot.

Dapivirine concentrations in rectal fluid and rectal tissue

- Table summarizing the concentration of dapivirine after single dose: at 30-60 min (visit 3), 120 min (visit 3), 24 h (visit 4), 48 h (visit 5) and 72 h (visit 6) after the time of dosing.
- Table summarizing the concentration of dapivirine (in rectal fluid only) after the first of multiple doses: at time of dosing (visit 7) and 24 h after time of dosing (visit 8).
- Table summarizing the concentration of dapivirine after multiple doses: at 30-60 min (visit 13), 120 min (visit 13), 24 h (visit 14), 48 h (visit 15) and 72 h (visit 16) after time of dosing.
- Individual concentration profiles, before and after dosing, overlaid on a single plot for each different sampling schedule.
- Plot of the median of dapivirine concentration in rectal fluid and rectal tissue over time, with vertical bars indicating 95% confidence interval. Separate plots for concentrations after single and after multiple dosing as well as overlaid on a single plot.

8.2.2 Elimination constant rate and terminal elimination half-life

To determine the terminal elimination half-life of dapivirine from blood, we will use the dapivirine concentrations from samples at 24 h, 48 h and 72 h after the time of dosing. To determine the terminal elimination half-life of dapivirine from rectal fluid and rectal tissue, for each participant we will use their concentration at 120 min after dosing and their concentration at either 24 h, 48 h or 72 h after dosing. Given the sampling schedule, only about half of the participants will have concentrations of rectal fluid and rectal tissue at two of these sampling time points available.

For the calculation of the terminal elimination half-life, for each participant, we will assume a mono-compartmental model, with the concentration at time t given by:

$$C(t) = a\, e^{-\beta t}$$

To estimate each participant's elimination rate constant (β), we will use linear regression on the log-transformed concentrations of dapivirine, with time (of sampling) as predictor, along with an indicator for samples obtained after multiple dosing. The resulting absolute value of the slope will correspond to the elimination rate constant β. The terminal elimination half-life for each participant will be calculated from the elimination rate constant as

$$t_{1/2} = \frac{\log(2)}{\beta}$$

We will summarize terminal elimination half-life ($t_{½}$) for blood, rectal fluid and rectal tissue, using descriptive statistics and will provide a 95% confidence interval, using a Student's-t distribution approximation.

9. SECONDARY ENDPOINTS

9.1 Acceptability

To assess acceptability of the study gel, we will focus on the ease of use and comfortability of the study product, as reported by participants, using information from the Exit Behavioral Survey. We will present results from the section B of the survey on Gel Acceptability, specifically the following two questions:

B1. Overall how easy or difficult was it to use the gel?

1. Very difficult
2. Difficult
3. Easy
4. Very easy

B2. Overall, how did it feel to have the gel inside you?

1. Very comfortable
2. Comfortable
3. Uncomfortable
4. Very uncomfortable

Results will be presented overall and by arm, with the number and percentage of participants in each category. To conduct formal statistical testing, collapsed categories will be created. For question B1, we will calculate the proportion of participants that responded “Easy” or “Very easy”. For question B2, we will calculate the proportion of participants that responded “Liked” or “Liked very much”. We will present the binomial proportions with exact 95% confidence intervals, overall and for each arm. Fisher’s Exact test will be used to test for differences between arms

Results will also be presented overall and by site, with no formal statistical testing.

9.2 Mucosal safety

Because the evaluations for safety focused on the rectal mucosal (proteomics, transcriptome, microflora, histology, tissue flow cytometry) include very specialized analyses, these will be described in a separate statistical analysis plan and performed separately.

10. SAFETY ANALYSES

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

10.1 Adverse Events and Deaths

See Section 8 for the description of the analyses on Adverse Events as the primary study endpoint related to Safety.

10.2 Laboratory Evaluations Results

10.2.1 Laboratory Evaluations
Laboratory results as collected on the Local Laboratory eCRF will be presented in tables by arm and visit and by site and visit. Summary statistics appropriate for the measurement scale will be used to describe AST (SGOT), ALT (SGPT), and creatinine values and severity grades. No formal statistical testing will be performed.

10.2.1 HIV Testing

Number and percentage of participants with a positive HIV test result as collected on the HIV Test Results eCRF will be presented in tables by arm. No formal statistical testing will be performed.

10.3 Other Safety Measures

10.3.1 Anorectal Exam

Anorectal exam results as collected on the Anorectal Exam eCRF will be presented in tables by arm and visit and by site and visit. Number and percentage of participants with any abnormal perianal examination, digital rectal examination, rectal mucosa from anoscopy, and sigmoidoscopy findings at each visit will be presented as well as the number and proportions of participants with specific findings. No formal statistical testing will be performed.

10.3.2 Concomitant Medications

A listing of all concomitant medications as collected on the Concomitant Medications Log eCRF will be presented, including, indication, date started and stopped, whether the medication is ongoing, frequency, route, dose, dose units, whether the medication was taken for an AE, and if so what AE(s). No formal statistical testing will be performed.