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MTN-025

A Phase 3B Open-Label Follow-on Trial to Assess the Continued Safety of and Adherence to a Vaginal Ring Containing Dapivirine in Women

- Statistical Analysis Plan v1.0 dated 15 April 2019
- Statistical Analysis Plan – Virology Addendum Analysis of Dapivirine Vaginal Ring-004 Clinical Trial MTN-025

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Statistical Center for HIV/AIDS Research and Prevention

STATISTICAL ANALYSIS PLAN

MTN-025

A Phase 3B Open-Label Follow-on Trial to Assess the Continued Safety of and Adherence to a Vaginal Ring Containing Dapivirine in Women

Microbicide Trials Network

Protocol Statistician:
Elizabeth R. Brown, ScD
Fred Hutchinson Cancer Research Center

SAP Version 1.0

April 15, 2019

SCHARP
Statistical Center for HIV/AIDS Research and Prevention

Approval Signature Page

MTN-025

Statistical Analysis Plan

A Phase 3B Open-Label Follow-on Trial to Assess the Continued Safety of and Adherence to a Vaginal Ring Containing Dapivirine in Women

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

15 April 2019
Date

Elizabeth Brown, ScD
MTN-025 Protocol Statistician
Fred Hutchinson Cancer Research Center

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1. LIST OF ABBREVIATIONS AND ACRONYMS

ACASI	audio computer-assisted self-interview
AE	adverse event
AIDS	acquired immunodeficiency syndrome
ALT	alanine transaminase
ARV	antiretroviral
ASPIRE	A Study to Prevent Infection with a Ring for Extended Use
AST	aspartate aminotransferase
CI	confidence interval
CRF	case report form
DAIDS	Division of AIDS, US National Institutes of Health
DPV	dapivirine
DVR	Dapivirine Vaginal Ring (dapivirine (25 mg) in a silicone elastomer vaginal matrix ring; Ring-004)
HIV	human immunodeficiency virus
HOPE	HIV Open-label Prevention Extension
ITT	intention to treat
MedDRA	Medical Dictionary for Regulatory Activities
MTN	Microbicide Trials Network
NGS	next generation sequencing
NL	network laboratory
NNRTI	non-nucleoside reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor
PI	protease inhibitor
PUEV	Product Use End Visit
PTID	Participant Identification Number
RTI	Reverse transcriptase inhibitor
SCHARP	Statistical Center for HIV/AIDS Research and Prevention
SDMC	Statistical Data Management Center
SOC	System Organ Class
STI	sexually transmitted infection

2. INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to provide a description of the statistical analyses of the MTN-025 (HOPE) trial that will be conducted to assess the safety, study product adherence, HIV-1 infection incidence, and drug resistance of the Dapivirine Vaginal Ring (dapivirine (25 mg) in a silicone elastomer vaginal matrix ring; Ring-004) (DVR) among former MTN-020 (ASPIRE) trial (Phase III) participants who are HIV-uninfected and not pregnant.

2.1 General Design Considerations

MTN-025 is a multi-site, open label extension trial of the DVR, inserted once every 4 weeks for the prevention of HIV-1 infection in healthy, sexually active HIV-1-uninfected women. The study enrolled women from 14 study sites in four African countries: Malawi (Blantyre, Lilongwe), Uganda (Kampala), South Africa [Cape Town, Durban (six sites), Johannesburg] and Zimbabwe [Harare (three sites)].

The primary focus of MTN-025 is the collection of additional adherence and safety data. Further, MTN-025 will examine incidence of HIV-1 infection and explore the way in which participants adopt this biomedical prevention method and incorporate it into the context of their everyday lives.

Participants enrolled into MTN-025 were divided into two populations: the main study population and the decliner population. Participants in the main study population had the choice of using the ring and provided all follow-up information for analysis. This population consisted of participants meeting the eligibility criteria described in Sections 5.2 and 5.3 of the protocol who consented to participate in follow-up visits and were enrolled, defined as having a valid enrollment date entered on the Enrollment CRF (Main study). Note that participants did not need to accept a vaginal ring at their enrollment visit in order to enroll into the MTN-025 main study population. The decliner population only provided baseline information and declined further follow-up. The decliner population consisted of former MTN-020 participants who met the eligibility criteria described in Sections 5.4 and 5.5 of the protocol but declined participation in the follow-up portion of the study and were not provided the DVR, defined as having a valid enrollment date on the Enrollment – Decliner Population CRF. Participants were considered enrolled into the main study population on the enrollment date on the Enrollment CRF or the enrollment date on the Enrollment – Decliner Population CRF.

Per Section 13.5 of the protocol, participation in the decliner population did not preclude later participation in the main study. Decliner population participants who later enrolled in the main study will be included in both populations and will be considered enrolled in the decliner and main study populations as of the enrollment date on the Enrollment – Decliner Population CRF and the Enrollment CRF, respectively.

The following is a protocol summary of the study.

Protocol Title:	A Phase 3B Open-Label Follow-on Trial to Assess the Continued Safety of and Adherence to a Vaginal Ring Containing Dapivirine in Women
Short Title:	HIV Open-label Prevention Extension (HOPE)
IND Sponsor:	International Partnership for Microbicides
Protocol Chair:	Jared Baeten, MD, PhD
Protocol Co-chair:	Nyaradzo M. Mgodi, MBChB, MMed Thesla Palanee-Phillips, PhD
Sample Size:	Former MTN-020 participants who are HIV-uninfected and not pregnant *Decliner Population: Former MTN-020 participants who decline participation in the main MTN-025 study and meet eligibility criteria as described in Sections 5.4 and 5.5 of the protocol*
Study Population:	Sexually active HIV-uninfected women, non-pregnant, 18-45 years of age
Study Sites:	Approved former MTN-020 sites
Study Design:	Phase 3B, open-label, multi-site trial
Study Duration:	Approximately 13 months of follow-up per participant with a projected accrual period of approximately 6 months at each site.

	Note: In an effort to provide women with the maximum ability to enter the MTN-025 trial, following the formal ~6-month study accrual period participants will continue to be enrolled throughout the duration of the trial, provided that at least 4 months of time on study is supported by the timeline. An adjusted (shortened) follow-up period will be employed for women who enroll after the formal accrual period.
Study Product:	Dapivirine Vaginal Ring (DVR)
Study Regimen:	Participants will receive a silicone elastomer vaginal matrix ring containing 25 mg of dapivirine to be replaced each month for a total period of 12 months of use. The study follow-up schedule will be monthly for the first three months, then quarterly thereafter (Figure 1), reflecting a transition to a more real-world type of follow-up (versus a clinical trial approach) that would be important for informing implementation.

2.2 Study Objectives and Endpoints

Primary Objectives:

1. Safety
   - To characterize the safety profile associated with the open label use of the dapivirine vaginal matrix ring (25 mg) in women
2. Study Product Adherence
   - To characterize adherence to the open label use of the dapivirine vaginal matrix ring (25 mg) in women

Primary Endpoints:

1. Safety
   a. Grade 2 adverse events (AEs) judged to be related to the dapivirine vaginal ring
   b. Grade 3 and higher AEs
   c. All serious AEs
2. Study Product Adherence
   a. Residual levels of dapivirine in returned vaginal rings
   b. Blood dapivirine levels

Secondary Objectives:

1. Incidence

- To assess incidence of HIV-1 infection

2. Drug Resistance

- To assess the frequency of HIV-1 drug resistance in women who acquire HIV-1 infection

Secondary Endpoints:

1. Incidence

- HIV-1 infection as measured by the protocol algorithm

2. Drug Resistance

- HIV-1 drug resistance mutations among participants who acquire HIV-1, as measured by standard genotype analysis and more sensitive methods to detect low frequency drug-resistant variants

2.3.1 Randomization

This is an open-label, follow-on trial. No randomization was done.

2.3.2 Blinding

This is an open-label and unblinded trial.

2.3.3 Sample Size and Power

Between 1000 and 2500 participants are expected to enroll in this study. The final number is dependent upon the proportion of ASPIRE participants who are eligible and choose to enroll into MTN-025, HOPE. Power is discussed with the primary analyses as appropriate.

3. GENERAL DATA ANALYSIS CONSIDERATIONS

3.1 Analysis Set(s)

This section describes the cohorts for primary and secondary analyses. These cohorts only include participants enrolled into the main study as defined in Section 2.1

Primary Cohort: The Primary Cohort includes all participants enrolled into the main study population.

Exposed Primary Cohort: This cohort differs from the Primary Cohort only in the exclusion of participants who never were provided a ring.

HIV Incidence and Adherence Cohort: This cohort differs from the Primary Cohort only in the exclusion of participants deemed to be HIV-1 RNA positive at the time of enrollment.

Exposed HIV Incidence and Adherence Cohort: This cohort differs from the Exposed Primary Cohort only in the exclusion of participants deemed to be HIV-1 RNA positive at the time of enrollment.

3.2 Statistical Analysis Issues

Although study procedures are in place to minimize the amount of missing values for primary and secondary endpoints, it is likely that missing data will occur. If specific analyses require rules for missing data, those will be specified in the statistical plan specific to that analysis.

4. INTERIM ANALYSIS AND DATA MONITORING COMMITTEE

No interim statistical analysis was planned or performed for MTN-025.

Three safety monitoring committee (SMC) reviews were conducted for MTN-025 in May 2017, November 2017, and May 2018. Open and closed reports were produced for all three SMC reviews.

5. GENERAL ANALYSIS METHODS

Descriptive statistics that will be used to summarize continuous variables are as follows: mean and standard deviation, median and interquartile range, quartiles, range, and number of missing data values. If applicable, continuous data that are skewed will be transformed to meet assumptions of normality. For categorical variables, descriptive statistics that will be used include the following: frequencies, relative frequencies, and the number of missing data values. Descriptive analyses summarizing baseline and follow-up data will be summarized overall, stratified by site and/or country, stratified by MTN-020 randomization arm (placebo vs. dapivirine), and stratified by provision of DVR (participants ever provided DVR vs. participants never provided DVR). The only exception is that analyses in the Exposed Primary Cohort and Exposed HIV Incidence and Adherence Cohorts will not stratify by provision of DVR (since these cohorts only include participants who were ever provided DVR). Line and box plots will be used, as appropriate, for longitudinal data representations. An alpha level of 0.05 will be used for all statistical tests unless otherwise specified.

A baseline measure is defined as either data collected at the Screening Visit (the visit window opens 56 days prior to the Enrollment visit and the visit code=1.00=100) or at the Enrollment Visit (visit code=2.00=200). If the same data is collected at the Screening Visit and the Enrollment Visit, then data collected at the Enrollment Visit will be used. See Appendix 1 for details regarding the MTN-025 visit windows.

Data from both regularly scheduled and unscheduled (referred to as interim) visits will be used in the analyses, as appropriate. Unless otherwise noted, data from interim visits will not contribute to summaries by visit but will contribute to summaries that are combined across all visits.

6. TRIAL PARTICIPANT DISPOSITION

6.1 Disposition of Participants

The disposition of trial participants will be presented in a CONSORT diagram and table. The measure, statistic and data source used to define each variable are described in the table below.

Measure	Statistic	Data Source
All participants enrolled in MTN-020	Number	Number of observations in Pre-Screening Outcome CRF Source: Pre-Screening Outcome CRF
All participants contacted (including Main Study Participants and Decliners)	Number and percentage	Was the participant contacted to participate in HOPE? (Q2) is 'yes' Source: Pre-Screening Outcome CRF
Main Study Participants screened	Number and percentage	Participant ID is not missing and does this participant meet all eligibility criteria (Q1) is not missing Source: Eligibility Criteria CRF
Main Study Participants eligible	Number and percentage	Does this participant meet all eligibility criteria (Q1) is 'yes' Source: Eligibility Criteria CRF

Main Study Participants not eligible	Number and percentage	Does this participant meet all eligibility criteria (Q1) is 'no' Source: Eligibility Criteria CRF
Main Study Participants enrolled into the study	Number and percentage	Enrollment date is not missing (Q5) Source: Enrollment CRF
Main Study Participants who were inappropriately enrolled	Number and percentage	Reason for termination (Q2) equals 'inappropriate enrollment' Source: Termination CRF
Main Study Participants who completed the study	Number and percentage	Reason for termination (Q2) equals 'scheduled exit visit/end of study' Source: Termination CRF
Reasons main study participants terminated early from the study	Number and percentage	Reason for termination (Q2) equals 'death', 'participant refused further participation', 'participant relocated, no follow-up planned', 'investigator decision', 'unable to contact participant', and 'other' Source: Termination CRF
Other reasons for termination (main study participants)	Number and percentage	Reason for termination (Q2) equals 'invalid ID due to duplicate screening/enrollment' and 'early study closure' Source: Termination CRF
Main Study Participants permanently discontinued from study product use	Number and percentage	Participant ID is not missing and response to: Was the participant instructed to resume study product use? (Q8) equals 'No - permanently discontinued' Source: Clinical Product Hold/Discontinuation Log CRF
Reasons for permanent discontinuation of study product (main study participants)	Number and percentage	Responses to: Why is study product being held? (Q2) among participants identified as permanently discontinued from study product use Source: Clinical Product Hold/Discontinuation Log CRF
Decliners screened	Number and percentage	Participant ID is not missing and does this participant meet all eligibility criteria as part of the decliner population (Q1) is not missing Source: Eligibility Criteria – Decliner Population CRF
Decliners eligible	Number and percentage	Does this participant meet all eligibility criteria as part of the decliner population (Q1) is 'yes' Source: Eligibility Criteria – Decliner Population CRF
Decliners not eligible	Number and percentage	Does this participant meet all eligibility criteria as part of the decliner population (Q1) is 'no' Source: Eligibility Criteria – Decliner Population CRF

Decliners enrolled into the study	Number and percentage	Enrollment date is not missing (Q2) Source: Enrollment – Decliner Population CRF
Decliners participants who later enrolled into main study	Number and percentage	Enrollment date is not missing (Q5) Source: Enrollment CRF and enrollment date is not missing (Q2) Source: Enrollment – Decliner Population CRF

6.2 Treatment Exposure

Not all participants accepted a ring and many participants first accepted a ring after enrollment. Treatment exposure is, therefore, defined as having been provided a ring at any time point prior to the current time. This is a time varying indicator, that is FALSE before a ring is first provided and true after.

6.3 Protocol Deviations

A table will be presented showing the number and type of protocol deviations, the number of deviations reported to local IRB/EC, and the number reported to DAIDS as a critical event based on the Protocol Deviation Log CRF.

7. BASELINE DATA

Unless otherwise specified, baseline characteristics will be summarized descriptively using appropriate summary statistics and reported across cohorts as described in Section 3.1 as well as for the decliner population, unless otherwise specified. No formal statistical testing will be performed.

7.1 Screening

The number of participants screened, enrolled and the reasons why participants were not enrolled (including reasons for ineligibility) will be summarized for all participants who were screened for enrollment into MTN-025. Separate summaries will be prepared for screening and enrollment into the main study population (i.e., Primary Cohort) and for screening and enrollment into the decliner population (see Section 6.1 Disposition of Participants for definitions).

7.2 Accrual

The activation dates, first and last enrollment dates, duration of accrual, eligible participants from ASPIRE, total contacted, total screened, total enrolled, and average enrolled per week will be summarized. The main study and decliner populations will be summarized separately.

7.3 Demographics

Baseline demographics (from items on the Demographics CRF) will include the following:

- participant age (calculated as INT((date of enrollment – date of birth)/365.25) or if date of birth unknown then “Age (Entered by Site)” from CRF)
- marital status (married/not married from item “Is the participant currently married?”)

- highest level of education [no schooling, primary school (partial and complete), secondary school (partial and complete), attended college or university]
- number of alcoholic drinks per week
- number of cigarettes per day
- how long did it take the participant to travel from home to the clinic today (less than 30 minutes, 30-60 minutes, 1-2 hours, greater than 2 hours, N/A)
- does the participant earn an income of her own (yes/no)
- how does she earn her income (formal employment, self-employment, and other)
- the number of times the participant has been pregnant
- the number of live births the participant has had
- whether the participant became pregnant since the end of ASPIRE (yes/no)
- if the participant became pregnant since the end of ASPIRE whether the participant was taking any measures to avoid falling pregnant (yes/no)
- religion (Christian/Muslim/other/none)
- number of times a week the participant attends religious services (more than once/once/less than once/never)
- how often the participant was worried that she would not have enough food in the past four weeks (never/rarely (once or twice)/sometimes (3-10 times)/often (more than 10 times)
- does the participant's household have the following:
    - electricity or solar panels (yes/no)
    - a radio (yes/no)
    - a cassette player (yes/no)
    - a television (yes/no)
    - a mobile telephone (yes/no)
    - a non-mobile telephone (yes/no)
    - a refrigerator (yes/no)
    - a table (yes/no)
    - a sofa (yes/no)
    - a bed (yes/no)
    - a CD or digital music player (yes/no)
    - a VCR/DVD player (yes/no)
    - a car (yes/no)
    - a motorcycle (yes/no)
    - a bicycle (yes/no).

7.4 Relationship and Sexual Risk Behaviors

Baseline relationship and sexual risk behaviors (from Baseline Behavior Assessment) will include the following:

- had a primary sex partner in the past 3 months (yes/no)
- primary sex partner the same partner when participant exited ASPIRE (yes/no)
- age of primary sex partner (years OR don't know)
- currently living with your primary sex partner (yes/no)
- primary sex partner provides participant with financial and/or material support (yes/no)
- primary sex partner knows participant has been offered to take part in the study (yes/no/not sure)
- primary sex partner knows participant has been offered to use a vaginal ring (yes/no/not sure)
- primary sex partner is circumcised (yes/no/don't know)
- primary sex partner HIV status (HIV positive/HIV negative/participant does not know)
- primary sex partner is taking ARVs (yes/no/don't know)
- in the past month did primary sex partner come to the study clinic (yes/no)
    - if primary partner came to the study clinic, did he come with the participant (yes/no)
    - did he receive counseling or other services from the study clinic (yes/no)
    - did he come to the study clinic for any other reason (yes/no)
- have you had the same primary sex partner for the last 3 months (yes/no)
- how many sex partners other than a primary sex partner have you had in the past 3 months
- in the past 3 months has your primary sex partner or ANY other current or previous partner ever:
    - slapped you, hit you with a fist or something else, or beaten you (yes/no)
    - kicked, dragged, pushed, pulled your hair, chocked or burnt you (yes/no)
- in the past 12 months has your primary sex partner or ANY other current or previous partner ever forced you to have sex by holding you down or hurting you (yes/no)
- in the past 3 months how many times in total have you had vaginal sex
- how many acts of vaginal sex did you have in the past 7 days
- how many acts of vaginal sex in the past 7 days was a male or female condom used
- during the last act of vaginal sex that you had, was a male and/or female condom used (male condom/female condom/both/none)
- in the past 3 months, how many times have you had anal sex
- during the last act of anal sex that you had was a male condom used (yes/no)
- in the past 12 months was getting HIV something you have thought about (never thought about/rarely thought about/thought about often)
- how worried are you that you might get HIV in the next 12 months (very worried/somewhat worried/not at all worried)
- how likely is it that you will become infected with HIV in the next 12 months (very unlikely/somewhat likely/very likely)
- how certain do you feel that you can protect yourself from getting infected with HIV (very uncertain/somewhat certain/very certain)
- how worried are you about having a vaginal ring inside of you every day for a year (very worried/somewhat worried/not at all worried)
- how much protection do you feel that the dapivirine ring can provide against HIV (the ring can provide a little protection/the ring can provide some protection/the ring can provide a lot of protection).

For the decliner population, a summary of reasons why a woman may have chosen not to participate in the study (quoted from Baseline Behavior Assessment) will include:

- you are not at risk for HIV (yes/no)
- it does not matter to you if you get HIV (yes/no)
- you are worried that the ring will harm your health (yes/no)

- the ring is not as good at preventing HIV as you thought (yes/no)
- you are worried people will think you are HIV positive (yes/no)
- you want to avoid side effects you experienced in ASPIRE (yes/no)
- you want to avoid side effects that you heard about in ASPIRE (yes/no)
- waiting time at the clinic (yes/no)
- having to return to the clinic frequently (yes/no)
- having to keep the ring inserted all the time (yes/no)
- having to keep the ring in during menses (yes/no)
- having to keep the ring in during sex (yes/no)
- you or your partner want to get pregnant (yes/no)
- having blood draws or other clinical procedures (yes/no)
- having to answer questions about your behavior during the study (yes/no)
- partner not supportive of study participation (yes/no)
- family not supportive of study participation (yes/no)
- other reason for choosing not to participate (yes/no)
- the main reason that you are not willing to participate in HOPE.

For the cohorts in 3.1 only, a summary of reasons why a woman may have chosen to participate in the study (quoted from Baseline Behavior Assessment) will include:

- to get tested for HIV (yes/no)
- to get counseling on reducing risk of HIV and STIs (yes/no)
- to help the community/to help fight the HIV epidemic (yes/no)
- because the ring can protect you against HIV (yes/no)
- to make it safer for you to have sex without condoms (yes/no)
- because this is the only or best way for you to get health care (yes/no)
- because you have friends who will probably participate in HOPE (yes/no)
- because you feel taken care of by the study staff (yes/no)
- because being in the study allows you to join social events at the clinic (yes/no)
- because being in the study helps you feel better about yourself (yes/no)
- because your study visits give you someone to talk to (yes/no)
- because the study visit reimbursement money is helpful (yes/no)
- other reason for choosing to participate (yes/no)
- the main reason that you are willing to participate in HOPE.

7.5 Baseline Abnormal Pelvic Exam Findings

For the cohorts described in Section 3.1, baseline abnormal pelvic exam findings will include the occurrence of findings shown on the Pelvic Exam CRF at Screening Visit.

7.6 Baseline Sexually Transmitted Infections (STIs)

For the cohorts described in Section 3.1, baseline sexually transmitted infections will include findings from the STI Test Results CRF at Screening Visit as follows: Syphilis Serology, Trichomonas Rapid Test, N. gonorrhoeae and C. trachomatis. The Syphilis result will be based on a reactive result to the Syphilis screening test plus a positive confirmatory test result. The number and percentage of participants experiencing each individual STI as well as any of the four STIs will be summarized.

7.7 Baseline Contraceptive Use

For the cohorts described in Section 3.1, baseline contraceptive use will include all potential responses to 'What method(s) of contraception/family planning is the participant currently using? Family Planning/Contraception Method' from the Family Planning CRF as follows:

- none
- spermicide
- sponge
- oral contraceptive birth control pills
- (Ortho Evra) – The Patch
- implants
- female condoms
- male condoms
- sterilization (tubal ligation/hysterectomy/laparoscopy/other surgical procedure that causes sterilization)
- diaphragm
- intrauterine device (IUD)
- injectable contraceptive - Depo
- injectable contraceptive - NET-EN
- injectable contraceptive - Cyclofem
- injectable contraceptive – Other
- natural methods such as the withdrawal or rhythm method
- sex with partner who had vasectomy
- emergency contraception
- other.

Baseline family planning or contraception methods will be defined as methods where the date regimen started (Family Planning CRF) is on or before the enrollment date (Enrollment CRF) and the date regimen stopped (Family Planning CRF) is on or after the enrollment date or missing.

7.8 Baseline Ring Insertion

For the cohorts described in Section 3.1, baseline ring insertion will summarize the number and percentage of participants who had a new ring inserted at their enrollment visit as recorded on the enrollment visit Ring Collection and Insertion CRF.

8. REPORTING OF FOLLOW-UP CHARACTERISTICS

Follow-up characteristics will be reported for all cohorts listed in Section 3.1. Unless otherwise specified, follow-up characteristics will be summarized descriptively using appropriate summary statistics. No formal statistical testing will be performed. Unless otherwise specified, follow-up characteristics summarized by study visit will be summarized at the Month 1, Month 2, Month 3, Month 6, Month 9, and Month 12 study visits.

8.1 Visit Retention

Visit retention will include the number and proportion of women who were expected, not expected, completed, not completed and missed and if missed, who terminated early by study visit. A plot showing retention by study month will be presented.

8.2 Study Termination

The reasons for study termination and time to trial discontinuation based on the Termination CRF will be presented in a table. Time to trial discontinuation is defined as the termination date (from Termination CRF) minus enrollment date (Enrollment CRF).

8.3 Self-reported Product Adherence and Acceptability

Data from the Ring Adherence CRF will be summarized as follows:

- number of participant-visits with the Ring Adherence Form
- participant-visits where the participant reported disclosing her ring use to her primary partner (yes/no/not applicable)
- participant-visits where the ring was never out
- participant-visits where the ring was out for more than 12 hours continuously
- number of times in total the participant had a vaginal ring out
- number of times a vaginal ring was out for more than 12 hours continuously
- longest number of days in a row the vaginal ring was out
- reasons the ring(s) were out
- reasons ring was removed by participant or clinician
- reasons ring came out on its own.

In addition, data from the Ring Collection and Insertion CRF will be summarized as follows:

- participant-visits with the Ring Collection and Insertion Form
- participant-visits used for the ring collection/insertion assessment
- participant-visits where the ring was in place at start of visit
- participant-visits where ring was not in place at start of visit
- number of days the ring was out prior to visit
- participant chose to use a new ring at this visit (yes/no/not applicable)
- participant accepted to receive the ring(s) on a quarterly schedule (yes/no) and reasons if no
- reasons that the participant opted to not use the ring at this visit
- ring provided at this visit (yes/no)
- reason ring not provided (participant on clinical hold, participant has been permanently discontinued from product, participant declined study ring, scheduled Product Use End Visit (PUEV), early termination, other)
- new ring inserted at this visit (yes/no)
- ring in place at the end of the visit (yes/no)
- reason ring not in place at end of visit (participant declined to have ring inserted at clinic visit, participant had to leave before ring could be inserted, other).

8.4 Product Hold/Discontinuation

The number and proportion of women experiencing a product hold, the total number of product holds, the reasons for product holds, and types of product hold resolution based on the Clinical Product Hold/Discontinuation Log CRF and presented in tables. In addition, a table describing the reasons for permanent discontinuation of study product will be presented.

8.5 Pregnancy Incidence and Outcomes

The number and proportion of women becoming pregnant during follow-up, incidence rates of pregnancy (number and person-years), frequencies of pregnancy outcomes, and frequencies of fetal/infant congenital anomalies will be summarized in tables based on the Pregnancy Outcome and Pregnancy Report and History CRFs.

8.6 Relationship and Sexual Risk Behaviors

Plots showing percentages of participants by study visit will be presented for the following relationship and sexual risk behaviors variables from the Behavior Assessment CRF:

- percentage of participants who had vaginal sex with primary sex partner in the past 3 months
- number of vaginal sex acts in the last 7 days
- last act of vaginal sex with a condom (yes- male condom/yes- female condom/both/none).

In addition, a table will be presented showing the number of anal sex acts in the past 3 months (none/1 or more) and last act of anal sex with a condom (yes/no), with both variables being taken from ACASI.

8.7 Social Impact

Social impacts will be summarized based on the Social Impact CRF for the following variables: self-reported type of social impact (e.g., personal relationships with family (excluding partner), personal relationships with partner, personal relationships with others, travel/immigration, employment, education, medical/dental, housing, other) and the type of impact on the participants' quality of life (minimal disturbance, moderate disturbance with no significant impact, major disturbance with significant impact).

8.8 Social Benefits

Social benefits will be summarized based on the Social Benefits CRF for the following variables: self-reported type of social benefit (e.g., pride about project participation, feeling better about oneself, education, housing, nutrition/food, improved communication, work, income, HIV testing, treatment of STIs, treatment of other illnesses, planning/contraception, preventative care services, staying HIV free, altruism, activities, peer support, new relationships, other), the number of people the social benefit involved, and the type of impact of the social benefit (minimal, moderate – no significant impact, major – significant impact).

8.9 Social Influences

Social influences will be summarized based on the Social Influences CRF for the following variables: number of people the participant talked to about HOPE other than clinic staff, relationship of the participant to those people, whether the person was male or female, whether the person participated in HOPE, how important the person's view of the ring was to the participant, and whether the person was in favor or against the participant using the ring.

8.10 Safety Laboratory Results

Safety laboratory results from the Laboratory Results CRF will be summarized from the Screening Visit through PUEV. Boxplots of local laboratory values will be generated for baseline values and for values measured over the course of the study. Each boxplot will show the 1st quartile, the median, and the 3rd quartile. Outliers, or values outside the boxplot, will also be plotted. If appropriate, horizontal lines representing boundaries for abnormal values will be plotted.

Laboratory measures will be included as follows: hemogram (hemoglobin, hematocrit, MCV, platelets, WBC), differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils) and serum chemistries [AST (SGOT), ALT (SGPT), and creatinine]. Severity grades will be summarized for hemoglobin, platelets, WBC, neutrophils, lymphocytes, AST (SGOT), ALT (SGPT), and creatinine.

8.11 Abnormal Pelvic Exam Findings

Follow-up abnormal pelvic exam findings will include the occurrence, per participant, of a finding at any time during follow-up, as shown on the Pelvic Exam CRF. Numbers and proportions of participants with specific types of pelvic exam findings occurring during follow-up will be presented in tables.

8.12 Adverse Experience

In addition to the primary safety endpoints, adverse experiences reported during follow-up are coded using MedDRA 21.1 software and will be summarized as follows:

1. The number and percentages of participants with AEs for each severity grade (mild, moderate, severe, potentially life-threatening, or death) for the highest severity grade per participant overall and by MedDRA 21.1 system organ class and preferred term.
2. The number and percentages of AEs for each level of relationship to study product (not related, related) for the most causal relationship per participant overall and by MedDRA 21.1 system organ class and preferred term.
3. The number and percentages of DAIDS expedited adverse experiences and/or serious adverse experiences (as defined by ICH Guidance E2A) by severity grade (mild, moderate, severe, potentially life-threatening, or death) for the highest severity grade per participant overall and by MedDRA 21.1 system organ class and preferred term.
4. The number and percentages of AEs by severity and relationship to study product.
5. The number and percentages of participants with an AE by severity and relationship to study product.

Separate tabulations will be created for AEs of special interest i.e. urogenital disorders and STIs. For this purpose, preferred terms may be grouped in a category of interest.

8.13 Sexually Transmitted Infections

Sexually transmitted infections during follow-up will include findings from the STI Test Results CRF at post-Enrollment visits as follows: Syphilis Serology, Trichomonas Rapid Test, N. gonorrhoeae and C. trachomatis. The Syphilis result will be based on a reactive result to the Syphilis screening test plus a positive confirmatory test result. The number and percentage of participants experiencing each individual STI as well as any of the four STIs will be summarized.

9. PRIMARY ENDPOINT ANALYSIS

9.1 Primary Safety Analysis

Objective: To characterize the safety profile associated with the open label use of the dapivirine vaginal matrix ring (25 mg) in women

Cohorts: Primary Cohort and Exposed Primary Cohort

Strata: Overall for both cohorts. Events among the Exposed Primary Cohort will be stratified by randomization arm from MTN-020 and time-varying treatment exposure (defined in 6.2, i.e., events that occurred prior to the participant first being provided a DVR vs. events that occurred after first DVR provision).

Outcomes:

- Grade 2 and higher adverse events (AEs) judged to be related to the dapivirine vaginal ring
- Grade 3 and higher AEs
- All serious AEs

Analysis Details: The analysis of adverse events (AEs) will be mainly descriptive in nature, summarizing the number and proportion of participants experiencing the three safety outcomes and the total number of outcomes.

9.2 Primary Adherence Analysis

For the Exposed HIV Incidence and Adherence Cohort, adherence in MTN-025 will be summarized according to residual drug levels in returned rings and will be based on average rate of dapivirine released (see description in Appendix 2). Throughout the study participants received feedback on adherence based on a scale derived from MTN-020 ("no protection", "low protection", "moderate protection", and "high protection") and the number and proportion of participants in each of these categories will be reported overall and by the following visits at which the ring was returned: Month 1, Month 2, Month 3, Month 6, Month 9 and Month 12. Median and mean residual dapivirine levels will be presented in figures and summary tables overall and by site by study month ring dispensed and by calendar month ring dispensed. Boxplots of residual dapivirine levels will also be presented overall and by study month ring dispensed.

10. SECONDARY ANALYSES – HIV INCIDENCE

10.1 HIV-1 Seroincidence

Objective: To estimate the incidence of HIV-1 seroconversion

Endpoint: HIV-1 antibody detection

Censoring: Follow-up time will be censored at the last negative HIV test or PUEV, whichever comes first

Cohorts: HIV Incidence and Adherence Cohort and Exposed HIV Incidence and Adherence Cohort

Strata: Overall and by MTN-020 randomization arm for both cohorts. The HIV Incidence and Adherence Cohort will be further stratified by having ever been dispensed a ring.

Definition of the HIV-1 Seroconversion Endpoint:

HIV-1 seroconversion as measured by antibody detection will be assessed according to the protocol to assess HIV-1 infection. All study sites performed HIV-1 testing per the algorithm in Appendix III of the MTN-025 Version 2.0 protocol for purposes of HIV-1 determination. In addition, the MTN Network Lab (NL) verified the results of HIV-1 testing performed at the study site laboratories for purposes of eligibility determination and primary outcome ascertainment as specified in the protocol.

In addition, an Endpoint Adjudication Committee (EAC) provided guidance on endpoint determination to the Protocol Team on an as-needed basis. See the MTN Manual of Operational Procedures (MOP) for detailed information on the composition, roles, and responsibilities of the EAC (www.mtnstopshiv.org).

For analysis, the HIV-1 seroconversion endpoint is defined as the time of first detection of HIV-1 antibodies, either by assay or, when assay results are inconclusive, as determination by the EAC. Time to seroconversion will be censored at the first of the last negative HIV-1 antibody test or PUEV for those participants who do not seroconvert at or before PUEV. If a participant acquires HIV-2, her follow-up time will be censored at the time of HIV-2 detection. Follow-up time will be based on the date of the enrollment visit to the endpoint or censoring as defined.

Analysis Details:

A table will be presented showing the number of participants enrolled, number who were not HIV-1 positive at enrollment, and number of participants with no follow-up time after enrollment, the number of participants with any follow-up time after enrollment, the number of participants who have HIV-1 seroconverted, total follow-up time (person-years), and the HIV-1 incidence rate and 95% exact confidence interval using Poisson distribution per 100 person-years.

10.2 Incidence of HIV-1 infection

Objective: To estimate the incidence HIV-1 infection

Endpoint: First of HIV-1 RNA or DNA detection or seroconversion

Censoring: Follow-up time will be censored at the last negative HIV-1 RNA or DNA test or PUEV, whichever comes first

Cohorts: HIV Incidence and Adherence Cohort and Exposed HIV Incidence and Adherence Cohort

Strata: Overall and by MTN-020 randomization arm for both cohorts. The HIV Incidence and Adherence Cohort will be further stratified by having ever been dispensed a ring.

Definition of the Primary HIV-1 Infection Endpoint:
The HIV-1 infection endpoint is defined as the time of first detection of HIV-1 RNA/DNA or antibodies if this occurs at or before the PUEV visit. If the participant does not acquire HIV-1, her infection time will be censored at the last negative HIV-1 test date at or before the scheduled PUEV. All participants who test positive for HIV-2 will have their HIV-1 infection time censored at the date of the test that determined HIV-2 infection. Follow-up time will be based on the date of the enrollment visit to the endpoint or censoring as defined.

Analysis Details:
A table will be presented showing the number of participants who are not HIV-1 positive at enrollment, and number of participants with no follow-up time after enrollment, the number of participants with any follow-up time after enrollment, the number of participants who were HIV-1 infected during the course of the study, total follow-up time (person-years), and the HIV-1 incidence rate and 95% exact confidence interval using Poisson distribution per 100 person-years.

10.3 Incidence of HIV-1 infection while exposed to ring

Objective: To assess HIV-1 infection rate while exposed to ring.

Endpoint: First of HIV-1 RNA or DNA detection or seroconversion

Censoring: Follow-up time will be censored at the last negative HIV-1 RNA or DNA test or PUEV, whichever comes first

Cohort: Exposed HIV Incidence and Adherence Cohort

Definition of exposure:

The exposure is acceptance of the ring. Participants are considered exposed starting at the date on which they were first provided a ring, as indicated by item 6, “Was a ring provided at this visit?” on the Ring Collection and Insertion CRF (Section 6.2).

Analysis Details:

Follow-up time will be classified as either exposed or unexposed per the definition of exposure. The incidence rate for the exposed (unexposed) group will be defined as the number of infections that occur while exposed (unexposed) divided by the total number of person-years of exposure (non-exposure). The time between a negative RNA test and positive RNA test will be counted as exposed if the participant accepts/has a ring during that time period. That time period will only be counted as unexposed if the participant never has a ring at any point in the time period. Incidence estimates with 95% exact confidence intervals using Poisson distribution will be presented for both groups.

10.4 Pre-defined Stratified Analyses

The HIV-1 incidence analyses defined in Sections 10.2 and 10.3 will be repeated in strata defined by baseline characteristics as follows:

1. MTN-020 randomization arm
2. Age (< 25 vs. >= 25 years old)
3. Country (South African sites vs. Non-South African sites)
4. Educational status (less than secondary school vs. secondary school or higher)
5. Marital status (married vs. non-married)
6. Baseline STI (a positive result for any of the following: chlamydia, gonorrhea, trichomoniasis, or syphilis vs. none)
7. Partner knowledge of ring use (primary partner knows participant is using vaginal ring vs. primary partner does not know/unsure)
8. Number of sex partners in the last 3 months (0-1 vs. 2+)

The measure, description and definition and/or data source used to define each stratum are described in the table below.

Measure	Description	Definition and/or Data Source
Age	Indicator variable for age in years categorized as < 25 and >= 25	For main study population: age calculated as INT((date of screening – date of birth)/365.25 or if date of birth unknown then age in years (“Age (Entered by Site)”) For decliner population: age calculated as INT((date of enrollment – date of birth)/365.25 or if date of birth unknown then age in years (“Age (Entered by Site)”) Source: Demographics, Participant Date of Visit, and Enrollment – Decliner Population CRFs
Country	Indicator variable for South Africa vs. non-South Africa site	Defined by the first three digits of the participant ID Source: Participant ID

Measure	Description	Definition and/or Data Source
Educational Status	Indicator variable for less than secondary school vs. secondary school or higher	"Highest level of education", "No Schooling", "Primary School, not complete", and "Primary school, complete" correspond to "less than secondary school", "Secondary school, not complete", "Secondary school complete", and "Attended college or university" correspond to "secondary school or higher" Source: Demographics CRF
Marital Status	Indicator variable for married vs. non-married	"Is the participant currently married?"(yes/no) Source: Demographics CRF
Baseline STI	Indicator variable for whether a positive result was observed at screening for any of chlamydia, gonorrhoea, trichomoniasis, or syphilis versus negative results for all STIs	"C. trachomatis" (negative/positive), "N. gonorrhoeae" (negative/positive), "Trichomonas Rapid Test" (negative/positive), "Syphilis screening test" (non-reactive/reactive) and "Syphilis confirmatory test" (negative/positive/indeterminate) Syphilis positive result = reactive on screening test and positive on confirmatory test; negative result = non-reactive on screening test or reactive on screening test and negative on confirmatory test; indeterminate confirmatory test results will be assessed and treated as a separate group, if appropriate. Source: STI Test Results CRF, visit = 1
Partner knowledge of ring use	Indicator variable for primary partner knows that the participant is using vaginal ring (yes) vs. primary partner does not know that participant is using vaginal ring (no)	"At any time in the past 3 months, have you had a primary sex partner? By primary sex partner we mean a man you have sex with on a regular basis, or who is your husband, or who you consider to be your main partner." (yes/no), "Does he know that you have been offered to use a vaginal ring as part of this study? (yes/no/not sure). If yes to both questions then yes, otherwise no. Not sure responses will be assessed and treated as a separate group, if appropriate. Source: Baseline Behavior Assessment CRF

Measure	Description	Definition and/or Data Source
Number of sex partners in the last 3 months	Indicator variable for 0-1 partners vs. 2 or more partners	"At any time during the past three months, have you had a primary sex partner?" and "How many sex partners other than a primary sex partner have you had in the past 3 months?" If the response to "At any time during the past three months, have you had a primary sex partner?" is "no" then the number of sex partners in the last 3 months equals the number reported for "How many sex partners other than a primary sex partner have you had in the past 3 months?" If the response to "At any time during the past three months, have you had a primary sex partner?" is "yes" then the number of sex partners in the last 3 months equals the number reported for "How many sex partners other than a primary sex partner have you had in the past 3 months?" plus 1. Source: Baseline Behavior Assessment CRF

In addition, incidence will be calculated within each stratum.

10.5 Drug Resistance

A summary table will be presented that shows the number of participants with a completed resistance test, a successful resistance test, a failed resistance test, an NNRTI resistant strain, an NRTI resistant strain, and a PI Major resistant strain. In addition, a line listing will be made that shows the HIV-1 viral load, MTN-020 randomization arm and the NNRTI, NRTI, PI major, and RTI other mutations (if applicable) among participants with a successful resistance test. Rates of drug resistance in women who HIV-1 seroconvert during the study will be estimated. A laboratory-developed population genotyping assay will be used to test for HIV-1 Protease and Reverse Transcriptase drug resistance mutations as interpreted by Stanford HIVdb v8.4. Additionally, a laboratory-developed sensitive next generation sequencing (NGS) assay will be used to test for HIV-1 Reverse Transcriptase drug resistance mutations. Mutations are identified by evaluation against the HXB2 reference sequence and defined as resistance-associated using the 2017 IAS-USA mutation table.

11. DOCUMENT HISTORY

Revision	Effective Date	Activity Description
0.3	June 15,2018	New document. New version and effective date issued.
1.0	April 15, 2019	Version 1.0 prepared in advance of database lock.

		1: Reformatted document using Statistical Analysis Plan Template TMP-0041. All section numbers below refer to section number in version 1.0. 2: Added the following: • Introduction (section 2) • "All participants enrolled in MTN-020" and "Decliners participants who later enrolled into main study" to disposition of participants (section 6.1) • definition of treatment exposure (section 6.2) • additional baseline demographics variables (section 7.3) • additional relationship and sexual risk behaviors variables (section 7.4) • summary of participants experiencing any STI at baseline (section 7.6) • definition of which contraceptives are considered to be in use at baseline (section 7.7) • baseline ring insertion summary (section 7.8) • definition of time to trial discontinuation (section 8.2) • summary of reasons for product holds and types of product holds (section 8.4) • summary of fetal/infant congenital anomalies (section 8.5) • summary of social benefits (section 8.8) • summary of social influences (section 8.9) • fifth bullet summarizing number and percentages of participants with an AE by severity and relationship to study product (section 8.12)

		• summary of sexually transmitted infections during follow-up (section 8.13) • description of analyses for Primary Adherence Analysis (section 9.2) • additional stratification variable of MTN-020 randomization arm (section 10.4) • mutation types, database used for interpreting HIV sequencing results for drug resistance analysis, NRTI mutations, and PI Major mutations (section 10.5). 3: Removed the following: • references to treatment arm (throughout) • unused abbreviations and acronyms (section 1) • software version information (section 5) • targets of accrual and plot of accrual by calendar month (section 7.2) • references to study retention (section 8.1) 4: Updated the following: • used bullet points instead of lists (throughout) • product details, description of study populations (section 2.1) • analysis method for protocol deviation (section 6.3) • analysis for product hold/discontinuation (section 8.4) • pregnancy incidence and outcomes (section 8.5) • adverse experience (section 8.12)

		• analysis cohort, strata, and analysis details for primary safety analysis (section 9.1) • analysis cohort and analysis method for primary adherence analysis (section 9.2) changed structure of Secondary HIV Incidence Analyses for clarity (section 10)

APPENDIX 1

MTN-025 Visit Windows FINAL (up until 15 Sep – full calendar)

Visit	Allowable/Target Visit Window Opens	Target Day	Target Visit Window Closes	Allowable Window Closes
V1 - Screening	N/A	No more than 56 days prior to Enrollment	N/A	
V2 - Enrollment	Day after Screening Visit	0	56 days after Screening Visit	
V3 - Monthly Visit Month 1	14 days prior to Target Day	28	13 days after Target Day	
V4 - Monthly Visit Month 2	14 days prior to Target Day	56	13 days after Target Day	
V5 - Quarterly Visit Month 3	14 days prior to Target Day	84	13 days after Target Day	69 days after Target Day
V6 - Quarterly Visit Month 6	14 days prior to Target Day	168	13 days after Target Day	69 days after Target Day
V7 - Quarterly Visit Month 9	14 days prior to Target Day	252	13 days after Target Day	69 days after Target Day
V8 - PUEV* (Month 12)	14 days prior to Target Day	336	13 days after Target Day	Study Completion
V9 - Study Exit/Termination**	13 days after actual date of PUEV	364 (28 days after actual date of PUEV)	13 days after Target Day	Study Completion

MTN-025 Visit Windows (16 Sep – 13 Oct)

Visit	Allowable/Target Visit Window Opens	Target Day	Target Visit Window Closes	Allowable Window Closes
V1 - Screening	N/A	No more than 56 days prior to Enrollment	N/A	
V2 - Enrollment	Day after Screening Visit	0	56 days after Screening Visit	

V3 - Monthly Visit Month 1	14 days prior to Target Day	28	13 days after Target Day	
V4 - Monthly Visit Month 2	14 days prior to Target Day	56	13 days after Target Day	
V5 - Quarterly Visit Month 3	14 days prior to Target Day	84	13 days after Target Day	69 days after Target Day
V6 - Quarterly Visit Month 6	14 days prior to Target Day	168	13 days after Target Day	69 days after Target Day
V7 - Quarterly Visit Month 9	14 days prior to Target Day	252	13 days after Target Day	41 days after Target Day
V8 - PUEV* (Month 11)	14 days prior to Target Day	308	13 days after Target Day	Study Completion
V9 - Study Exit/Termination**	13 days after actual date of PUEV	336 (28 days after actual date of PUEV)	13 days after Target Day	Study Completion

MTN-025 Visit Windows (14 Oct – 10 Nov)

Visit	Allowable/Target Visit Window Opens	Target Day	Target Visit Window Closes	Allowable Window Closes
V1 - Screening	N/A	No more than 56 days prior to Enrollment	N/A	
V2 - Enrollment	Day after Screening Visit	0	56 days after Screening Visit	
V3 - Monthly Visit Month 1	14 days prior to Target Day	28	13 days after Target Day	
V4 - Monthly Visit Month 2	14 days prior to Target Day	56	13 days after Target Day	
V5 - Quarterly Visit Month 3	14 days prior to Target Day	84	13 days after Target Day	69 days after Target Day
V6 - Quarterly Visit Month 6	14 days prior to Target Day	168	13 days after Target Day	69 days after Target Day
V7 - Quarterly Visit Month 9	14 days prior to Target Day	252	13 days after Target Day	13 days after Target Day
V8 - PUEV* (Month 10)	14 days prior to Target Day	280	13 days after Target Day	Study Completion
V9 - Study Exit/Termination**	13 days after actual date of PUEV	308 (28 days after actual date of PUEV)	13 days after Target Day	Study Completion

MTN-025 Visit Windows (11 Nov – 8 Dec)

Visit	Allowable/Target Visit Window Opens	Target Day	Target Visit Window Closes	Allowable Window Closes

V1 - Screening	N/A	No more than 56 days prior to Enrollment	N/A	
V2 - Enrollment	Day after Screening Visit	0	56 days after Screening Visit	
V3 - Monthly Visit Month 1	14 days prior to Target Day	28	13 days after Target Day	
V4 - Monthly Visit Month 2	14 days prior to Target Day	56	13 days after Target Day	
V5 - Quarterly Visit Month 3	14 days prior to Target Day	84	13 days after Target Day	69 days after Target Day
V6 - Quarterly Visit Month 6	14 days prior to Target Day	168	13 days after Target Day	69 days after Target Day
V7 - Quarterly Visit Month 9 (PUEV)	14 days prior to Target Day	252	13 days after Target Day	Study Completion
V8 - Study Exit/Termination**	13 days after actual date of PUEV	280 (28 days after actual date of PUEV)	13 days after Target Day	Study Completion

MTN-025 Visit Windows (9 Dec – Jan 5)

Visit	Allowable/Target Visit Window Opens	Target Day	Target Visit Window Closes	Allowable Window Closes
V1 - Screening	N/A	No more than 56 days prior to Enrollment	N/A	
V2 - Enrollment	Day after Screening Visit	0	56 days after Screening Visit	
V3 - Monthly Visit Month 1	14 days prior to Target Day	28	13 days after Target Day	
V4 - Monthly Visit Month 2	14 days prior to Target Day	56	13 days after Target Day	
V5 - Quarterly Visit Month 3	14 days prior to Target Day	84	13 days after Target Day	69 days after Target Day
V6 - Quarterly Visit Month 6	14 days prior to Target Day	168	13 days after Target Day	41 days after Target Day
V7 - Quarterly Visit Month 8 (PUEV)	14 days prior to Target Day	224	13 days after Target Day	Study Completion
V8 - Study Exit/Termination**	13 days after actual date of PUEV	252 (28 days after actual date of PUEV)	13 days after Target Day	Study Completion

MTN-025 Visit Windows (6 Jan – 2 Feb)

Visit	Allowable/Target Visit Window Opens	Target Day	Target Visit Window Closes	Allowable Window Closes
V1 - Screening	N/A	No more than 56 days prior to Enrollment	N/A	
V2 - Enrollment	Day after Screening Visit	0	56 days after Screening Visit	
V3 - Monthly Visit Month 1	14 days prior to Target Day	28	13 days after Target Day	
V4 - Monthly Visit Month 2	14 days prior to Target Day	56	13 days after Target Day	
V5 - Quarterly Visit Month 3	14 days prior to Target Day	84	13 days after Target Day	69 days after Target Day
V6 - Quarterly Visit Month 6	14 days prior to Target Day	168	13 days after Target Day	13 days after Target Day
V7 - Quarterly Visit Month 7 (PUEV)	14 days prior to Target Day	196	13 days after Target Day	Study Completion
V8 - Study Exit/Termination**	13 days after actual date of PUEV	224 (28 days after actual date of PUEV)	13 days after Target Day	Study Completion

MTN-025 Visit Windows (3 Feb – 2 Mar)

Visit	Allowable/Target Visit Window Opens	Target Day	Target Visit Window Closes	Allowable Window Closes
V1 - Screening	N/A	No more than 56 days prior to Enrollment	N/A	
V2 - Enrollment	Day after Screening Visit	0	56 days after Screening Visit	
V3 - Monthly Visit Month 1	14 days prior to Target Day	28	13 days after Target Day	
V4 - Monthly Visit Month 2	14 days prior to Target Day	56	13 days after Target Day	
V5 - Quarterly Visit Month 3	14 days prior to Target Day	84	13 days after Target Day	69 days after Target Day
V6 - Quarterly Visit Month 6 (PUEV)	14 days prior to Target Day	168	13 days after Target Day	Study Completion
V7 - Study Exit/Termination**	13 days after actual date of PUEV	196 (28 days after actual date of PUEV)	13 days after Target Day	Study Completion

MTN-025 Visit Windows (3 Mar – 30 Mar)

Visit	Allowable/Target Visit Window Opens	Target Day	Target Visit Window Closes	Allowable Window Closes
V1 - Screening	N/A	No more than 56 days prior to Enrollment	N/A	
V2 - Enrollment	Day after Screening Visit	0	56 days after Screening Visit	
V3 - Monthly Visit Month 1	14 days prior to Target Day	28	13 days after Target Day	
V4 - Monthly Visit Month 2	14 days prior to Target Day	56	13 days after Target Day	
V5 - Quarterly Visit Month 3	14 days prior to Target Day	84	13 days after Target Day	41 days after Target Day
V6 - Quarterly Visit Month 5 (PUEV)	14 days prior to Target Day	140	13 days after Target Day	Study Completion
V7 - Study Exit/Termination**	13 days after actual date of PUEV	168 (28 days after actual date of PUEV)	13 days after Target Day	Study Completion

MTN-025 Visit Windows (31 Mar – 27 Apr)

Visit	Allowable/Target Visit Window Opens	Target Day	Target Visit Window Closes	Allowable Window Closes
V1 - Screening	N/A	No more than 56 days prior to Enrollment	N/A	
V2 - Enrollment	Day after Screening Visit	0	56 days after Screening Visit	
V3 - Monthly Visit Month 1	14 days prior to Target Day	28	13 days after Target Day	
V4 - Monthly Visit Month 2	14 days prior to Target Day	56	13 days after Target Day	
V5 - Quarterly Visit Month 3	14 days prior to Target Day	84	13 days after Target Day	
V6 - Quarterly Visit Month 4 (PUEV)	14 days prior to Target Day	112	13 days after Target Day	Study Completion
V7 - Study Exit/Termination**	13 days after actual date of PUEV	140 (28 days after actual date of PUEV)	13 days after Target Day	Study Completion

MTN-025 Visit Windows (28 Apr – 25 May)

Visit	Allowable/Target Visit Window Opens	Target Day	Target Visit Window Closes	Allowable Window Closes

V1 - Screening	N/A	No more than 56 days prior to Enrollment	N/A	
V2 - Enrollment	Day after Screening Visit	0	56 days after Screening Visit	
V3 - Monthly Visit Month 1	14 days prior to Target Day	28	13 days after Target Day	
V4 - Monthly Visit Month 2	14 days prior to Target Day	56	13 days after Target Day	
V5 - Quarterly Visit Month 3 (PUEV)	14 days prior to Target Day	84	13 days after Target Day	Study Completion
V6 - Study Exit/Termination**	13 days after actual date of PUEV	112 (28 days after actual date of PUEV)	13 days after Target Day	Study Completion

**PUEV must occur prior to completion of SEV.*
***SEV should not occur less than 2 weeks after PUEV.*

APPENDIX 2

Specifications drug release rate calculations

1) For each ring, we need the date it was dispensed (from Pharmacy Ring Dispensation CRF) and date it was returned (from Vaginal Ring Tracking CRF). From this information, create 4 variables per ring (record):
   a) Date of ring dispensation
   b) Date of ring return
   c) Amount of dapivirine dispensed: manufacturing lot level – residual drug level
   d) Days with ring = date of ring return – date of ring dispensation

2) To link the data with endpoints that are measured on the visit schedule, we will need to estimate the amount of drug released between scheduled visits. From Step 1, we know the average amount of drug released from rings on each day of follow-up. Based on that we can calculated the average amount of drug released daily between scheduled follow-up visits. In the majority of cases, ring dispensation and return dates will align perfectly with HIV testing dates, with one ring dispensed and returned per visit. However, there are cases where multiple rings are dispensed and returned, or a participant returns or obtains a new ring at two consecutive scheduled visits and also at some visit in between. A general formula that will work for all visits (indexed by k) and all rings (indexed by j) for a participant is as follows:

$$r_k = \frac{\sum_{j=1}^{J} I_{jk} \frac{d_j}{t_j^{(r)} - t_j^{(d)}} \left[\min\left(v_k, t_j^{(r)}\right) - \max\left(v_{k-1}, t_j^{(d)}\right)\right]}{v_k - v_{k-1}},$$

where
$I_{jk} = 1$ if $v_{k-1} \leq t_j^{(d)} < v_k \vee v_{k-1} < t_j^{(r)} \leq v_k \vee \left(t_j^{(d)} < v_{k-1} \wedge t_j^{(r)} > v_k\right)$, 0 otherwise
r_k is the rate per day of dapivirine release for each expected visit window denoted by the visit code using data from the time period from the previous up to the visit shown.
d_j is the amount of dapivirine released from the ring = manufacturing lot level – measured value from lab,
$t_j^{(d)}$ is the time in days since randomization at which ring j was dispensed,
$t_j^{(r)}$ is the time in days since randomization at which ring j was returned, and
v_k is the time in days since randomization at which the visit is scheduled, $v_0 = 0$

The numerator represents the total amount of DPV released between v_{k-1} and v_k and should be saved as well.

Exceptions:
- Time on product hold should have a rate = 0.
- If a ring was returned, but it's value is missing, the principal of last observation carried forward should be used.
- If the participant failed to return a ring, the dapivirine released during the time she should have had that ring should be zero.
- All visits at which a ring was not tested due to it being destroyed should be coded as missing.

INTERNATIONAL PARTNERSHIP FOR MICROBICIDES

Statistical Analysis Plan

Virology Addendum Analysis of Dapivirine Vaginal Ring-004 Clinical Trial MTN-025

(Phase IIIb Clinical Trial)

Version 1.0

International Partnership for Microbicides
8405 Colesville Road, Suite 600
Silver Spring, MD 20910 USA

APPROVAL SIGNATURE PAGE

MTN-025 Virology Addendum – Statistical Analysis Plan

I have read the above referenced Statistical Analysis Plan and approve its contents.

DocuSigned by:
John Steytler
Signer Name: John Steytler
Signing Reason: I approve this document
Signing Time: 28-May-2021 | 07:27:30 EDT
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John Steytler, MBChB
Head of Pharmacovigilance and Clinical Development
International Partnership for Microbicides

DocuSigned by:
Ben van Baelen
Signer Name: Ben van Baelen
Signing Reason: I approve this document
Signing Time: 30-May-2021 | 23:33:42 PDT
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Statistical consultant
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Signer Name: Neliette van Niekerk
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APPROVAL SIGNATURE PAGE

MTN-025 Virology Addendum – Statistical Analysis Plan

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ABBREVIATIONS

CSR	Clinical Study Report
DNA	Deoxyribonucleic acid
DVR-004	Dapivirine Vaginal Ring-004
EFV	Efavirenz
ETR	Etravirine
FC	Fold-change
HIV-1	Human immunodeficiency virus type 1
HIVdb	HIV Drug Resistance Database
IC_{50}	50% inhibitory concentration
IP	Investigational product (ie, the DVR-004 and placebo ring)
IPM	International Partnership for Microbicides
MTN	Microbicide Trials Network
NGS	Next generation sequencing
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
PCR	Polymerase chain reaction
PI	Protease inhibitor
RAM	Resistance-associated mutation
RNA	Ribonucleic acid
RPV	Rilpivirine
RT	Reverse transcriptase
SAP	Statistical Analysis Plan
STI	Sexually transmitted infection
T&L	Tables and Listings
UMI	Unique molecular identifier
USA	United States of America

TABLE OF CONTENTS

1. INTRODUCTION

This document serves as the Statistical Analysis Plan (SAP) for the supplementary virology analysis of the Dapivirine Vaginal Ring-004 (DVR-004) Phase IIIb open-label extension trial, MTN-025. Specifically, this document describes the process for identification of samples and statistical methods that will be used to analyze the data resulting from retrospective resistance testing using a unique molecular identifier (UMI) next generation sequencing (NGS) methodology[1-3], as well as phenotypic susceptibility analysis recombinant retroviral system[4].

There are several advantages of the NGS method employed in this analysis over standard NGS methodologies. The current methodology uses UMI 'barcodes' built into polymerase chain reaction (PCR) primers, which allows sequences originating from the same human immunodeficiency virus type 1 (HIV-1) ribonucleic acid (RNA) template to be grouped and aligned. Building consensus sequences from these alignments provides improved control of PCR and sequencing error, PCR bias, and PCR recombination. The number of UMI with consensus sequences allows the depth of sequencing to be calculated based on the number of HIV-1 RNA templates sampled, which can in turn be used to estimate the sensitivity of the individual sample analysis using an adaptation of the Clarke and Carbon equation

$[P=1-(1-f)^N$

where P = probability, N = number of sequences, f = fraction of sequences with variant][5,6] to obtain the proportion of a minority species that can be detected with 95% confidence. The assay has been validated to detect resistance-associated mutations (RAMs) down to 1% prevalence, subject to the number of consensus sequences (UMIs) observed. Despite the robust nature of the assay and the analysis of its results, the NGS analysis should still be considered as exploratory. Of note, clinical interpretation of these data remains challenging. In addition to technical challenges, there is an absence of cross-laboratory standardization of outputs and of accredited means for external quality assessment[7,8]. In the absence of resolution of these issues, the clinical relevance of the increased sensitivity for detection of resistance-associated mutations using NGS remains unknown.

Templates for all tables and listings that will be used to present the virology results are also provided. These analyses will be described in an addendum to the MTN-025 Clinical Study Report (CSR).

Table shells and listing shells that will accompany the analyses are provided in a separate document of mock tables and listings (T&L) (Appendix 1). This document is viewed as supporting material for the analyses and will not require signature approval if formatting changes are made.

2. OBJECTIVE

The primary purpose of this analysis is to further address, with supplemental information, the secondary objective for trial MTN-025:

- To assess the frequency of HIV-1 drug resistance in women who acquire HIV-1 infection while using the DVR-004.

Specifically, the analyses will facilitate the description of:

- the prevalence of all variants detectable to a minimum prevalence of 1%, 5%, or 10% in reverse transcriptase (RT) at seroconversion using NGS.
- the fold-change (FC) in phenotypic susceptibility of virus at seroconversion to dapivirine, efavirenz, etravirine, nevirapine, and rilpivirine relative to a wild-type control virus.
- the findings in relation to the population-based genotyping results described in the MTN-025 CSR Version 1.0, dated 20 April 2020, and the International Partnership for Microbicides (IPM) Phase III Clinical Virology Report Version 4.0.

2.1. Endpoints

Primary Endpoints:

- The presence of non-nucleoside reverse transcriptase inhibitor (NNRTI) RAMs, including minority species RAMs at seroconversion, using NGS.
- Phenotypic susceptibility of virus to dapivirine and the approved NNRTIs: efavirenz, etravirine, nevirapine, and rilpivirine, measured at seroconversion.

Secondary Endpoint:

- Comparison of specific NNRTI RAM detection using population-based genotyping and NGS.

Exploratory Endpoints:

- Correlation between phenotypic susceptibility findings and population-based genotype.

2.2. Assessments of Endpoints

Primary Endpoints Assessed by:

- Determination of the prevalence of all variants reported from NGS tests with sensitivity to detect viruses present at ≥ 10.0%, ≥ 5.0%, and ≥ 1.0% prevalence in HIV-1 RT at seroconversion.
- Determination of the 50% inhibitory concentration (IC_{50}) and FC in phenotypic susceptibility of virus at seroconversion to dapivirine, efavirenz, etravirine, nevirapine, and rilpivirine relative to a wild-type control.

Secondary Endpoint Assessed by:

- Summarizing numbers and prevalence of additional NNRTI RAMs observed using NGS compared with population-based genotyping, within the limits of the NGS sequence region (reverse transcriptase codons 81 to 149 and 152 to 212).

Exploratory Endpoints Assessed by:

- Summarizing viral FC in susceptibility to dapivirine by mutation patterns determined using population-based genotyping assessment.
- Comparison of IC_{50} values between viruses with known NNRTI RAMs and those with wild-type genotypes.

3. ANALYSIS POPULATIONS

3.1. Exposed Primary Population

The exposed primary population includes all trial participants who were enrolled from the parent trial, MTN-020 and were provided with at least one DVR-004.

3.2. Virology Population

The virology population includes all participants in the exposed primary population who experienced seroconversion (either using HIV-1 Rapid test result algorithms or determined to have been HIV-1 infected while using the DVR-004 based on reverse sequential HIV-1 RNA PCR testing) during investigational product (IP) use except:

- Participants who were deemed to be HIV-1 RNA positive at the time of enrolment.
- Participants who seroconverted after last product use (ie, at the Exit Visit) and were not HIV-1 RNA positive at the last product use visit, ie, were infected after they discontinued product use.

The virology population will be the main focus of this analysis. Available data for any participants infected with HIV-1 prior to enrolment or after last product use will be listed and described separately. Phenotypic analyses will be performed only on samples obtained at seroconversion during DVR-004 use.

4. GENERAL STATISTICAL CONSIDERATIONS

4.1. Analysis Software

Statistical analyses will be conducted by an external service provider of IPM and will be reviewed by IPM and the University of Pittsburgh Virology Laboratory. The analyses will be performed using SAS® for Windows or Linux/Unix (Version 9.2 or higher, SAS Institute Inc., Cary, North Carolina, United States of America [USA]).

4.2. Planned Subgroups

As numbers are small, there are no plans to perform analyses in subgroups. Should individual outlier results be observed, additional relevant characteristics may be described from the MTN-025 CSR listings or listings from this addendum.

4.3. Data Used for Analysis

Next generation sequencing will be attempted for all available seroconversion samples from all participants in the virology population. Results showing sensitivity to detect viruses present only at >10.0% prevalence (ie, with detection of < 28 UMI) will be summarized, although, due to inadequate sensitivity, these results will not be used to infer the absence of NNRTI RAMs. Analyses will be performed with cut-offs set to include the detection of minority species present in at least 10.0%, 5.0%, or 1.0% of UMI.

Results showing sensitivity to detect only minority species present with > 10.0% prevalence (ie, UMI < 28) will be described individually.

Phenotypic susceptibility and NGS will be performed on plasma samples taken at the time of HIV-1 seroconversion ± 14 days. Any results from other time points will be listed and may be discussed separately, but not included in summary analyses.

4.4. Methods for Handling Missing Data

While every effort will be made to minimize the amount of missing data, as this is a retrospective analysis, some degree of missing data associated with sample availability and participant consent, is expected. Numbers of missing analyses will be provided along with numbers of participants included in the analysis, and tests conducted with and without successful outcomes.

If the date of the last IP exposure (ie, last ring removal) is incomplete or missing, the following imputation will be made:

- when only the day is missing, impute with min (last ring insertion date + 28 days, last day of the month), else
- impute with last ring insertion date + 28 days.

This should be added in a footnote in the applicable tables/listings. If the date itself should be printed, the original (not the imputed) date should be printed.

5. ANALYSIS OF DEMOGRAPHIC AND BASELINE CHARACTERISTICS

As numbers are small, the demographic and baseline characteristics of participants in the virology population will not be described. The overall population characteristics are available in the MTN-025 CSR Version 1.0, dated 20 April 2020.

6. ANALYSIS OF GENOTYPING DATA

Antiretroviral drug NNRTI RAMs in virus from women who acquire HIV-1 while using the DVR-004 will be determined by means of NGS. (Population-based genotypes are reported in the MTN-025 CSR Version 1.0, dated 20 April 2020 and the IPM Phase III Clinical Virology Report, Version 4.0.) Next generation sequencing provides the supplementary, exploratory results primarily addressed in the current SAP.

6.1. Definition of Resistance-Associated Mutations

Summary analyses of NNRTI RAMs will be based on mutations identified in the Stanford HIV Drug Resistance Database (HIVdb) Version 8.4 (dated 16 June 2017, https://hivdb.stanford.edu/)[9]. The NNRTI RAMs with scores in the Stanford HIVdb algorithm Version 8.4 within the range of the NGS test include:

A98G, L100I, L100V, K101E, K101H, K101P, K103H, K103N, K103S, K103T, V106A, V106M, V108I, E138A, E138G, E138K, E138Q, E138R, V179D, V179E, V179F, V179L, V179T[a], Y181C, Y181F, Y181G, Y181I, Y181S, Y181V, M184I[a], Y188C, Y188F, Y188H, Y188L, G190A, G190C, G190E, G190Q, G190S, G190T, G190V (Appendix 2).

In addition, a summary of all amino acid variants from the reference sequence will be provided for both sequencing methodologies.

6.2. Population-based Sequencing

Population-based sequencing results at seroconversion have been described in the MTN-025 CSR (MTN-025 CSR Version 1.0, dated 20 April 2020) and in the IPM Phase III Clinical Virology Report, Version 4.0; individual participant results are provided in the listings associated with the MTN-025 CSR.

The population-based genotyping sequences and resistance predictions will also be used to examine consistency with the NGS findings and the phenotypic susceptibility results. The reference sequence for the population-based sequencing analysis was the Stanford HIVdb consensus subtype B sequence.

6.3. Next Generation Sequencing

For NGS, the wild-type subtype B sequence, HXB2 (accession number K03455-1), will be used as reference. In the region sequenced, the only difference from the Stanford HIVdb consensus subtype B sequence is at codon 122 (HXB2: K122; Stanford: E122 respectively); K122 (or E122K) and E122 (or K122E) will be considered to be polymorphisms not related to NNRTI resistance in the analysis. The sensitivity for each NGS determination indicates the required prevalence for changes from the reference sequence to be detectable in the sample. The prevalences required are determined based on the statistical consideration of requiring at least 298 UMI for sensitivity to detect a 1.0% minority, 58 UMI for a 5.0% minority and 28 UMI for a 10.0% minority and will be summarized in tabulations. Samples with results showing less than 298, 58 or 28 UMI will be censored from each analysis performed to sensitivities to detect 1.0%, 5.0% and 10.0% minority species, respectively.

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[a] These mutations do not have a resistance score when present alone; V179T with Y181C and M184I with K101E contribute to the score as a combination.

Frequency tabulations per minimum detectable prevalence (1.0%, 5.0%, 10.0%), as well as any additional NNRTI RAMs observed when results indicate UMI < 28, will be presented showing the percentage of participants with an amino acid change from reference at each position of the sequenced RT region.

All NGS results will be listed relative to the reference virus' amino acid sequence together with percent prevalence in the sample.

Frequency tabulations will be created per sensitivity cut-off (1.0%, 5.0%, 10.0%) for:

- The number (and percentage) of participants who had a resistance test performed.
- The number (and percentage) of participants with each individual NNRTI RAM.
- The number (and percentage) of participants with 0, 1, 2 or ≥ 3 mutations by class (NNRTI).
- The number (and percentage) of participants with combinations of specified NNRTI RAMs.

6.4. Phenotypic Susceptibility

Phenotyping data will be obtained for virus from women with HIV-1 seroconversion and who had a seroconversion sample with sufficient HIV-1 RNA for analysis.

Recombinant HIV-1 will be generated containing cloned RT from the participant's HIV-1 RNA from plasma that was sampled at the time of HIV seroconversion. Recombinant viruses will be tested in the TZM-bl cell line for susceptibility to dapivirine as well as to the NNRTIs: efavirenz, etravirine, nevirapine and rilpivirine. A wild-type recombinant virus with an anonymized HIV-1 RT amplicon from South Africa with no RT resistance-associated mutations cloned into an xxLAI vector[4] will be used to ensure values remain within acceptable limits.

For each NNRTI, susceptibility will be reported as the half maximal inhibitory concentration (IC_{50}). Fold-change resistance will be calculated for each individual NNRTI as:

$$FC = IC_{50}/IC_{50control}$$

- with $IC_{50control}$ = appropriate wild-type virus reference values.
- when more than one IC_{50} value is available for a sample (replicate test results), the geometric mean IC_{50} value of all replicates will be taken prior to the FC calculation.

Descriptive statistics of IC_{50} and FC values will be presented for each NNRTI and will include geometric mean and standard deviation, median, quartiles, range, and number of observations.

Additionally, descriptive statistics (geometric mean and standard deviation) of NNRTI FCs will be presented by NNRTI RAM pattern.

All individual participant data (IC_{50}, FC) will be listed. Individual IC_{50} determinations and FCs will be averaged using geometric means for each virus when multiple determinations are available.

7. REFERENCES

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10. Wensing AM, Calvez V, Ceccherini-Silberstein F, Charpentier C, Günthard HF, Paredes R, et al. 2019 update of the drug resistance mutations in HIV-1. Topic Antivir Med 2019, 27(3):111-121.

8. APPENDICES

8.1. Appendix 1 – Tables and Listings Titles

Tables

Table number	Title
14.3.9	**Next Generation Sequencing (NGS) and Phenotypic Susceptibility Analysis**
14.3.9.1	**Accountability**
14.3.9.1.1	Sample Availability
14.3.9.2	**Summary of NGS and Paired Population-based Genotype Analysis**
14.3.9.2.1	Summary of Number of Participants with an Amino Acid Change from Reference per Residue in the RT Region Among HIV-1 Seroconversions using Next Generation Sequencing with Paired Population-Based Genotypes
14.3.9.2.2	Summary of Numbers and Combinations of HIV-1 NNRTI Resistance-associated Mutations Among HIV-1 Seroconversions using Next Generation Sequencing with Paired Population-Based Genotypes
14.3.9.3	**Phenotypic Susceptibility Analysis**
14.3.9.3.1	Summary of Phenotypic Susceptibility to Dapivirine and Other NNRTIs by Population-based Genotype
14.3.9.4	**Summary of Population-Based Genotype Analysis (All Seroconversion Data)**
14.3.9.4.1	Summary of Number of Participants with an Amino Acid Change from Reference per Residue in the RT Region Among HIV-1 Seroconversions using Population-Based Genotyping

Listings

Listing number	Title
16.2.8.20	**Next Generation Sequencing and Phenotypic Data**
16.2.8.20.1	Next Generation Sequencing: Listing of All Available Data
16.2.8.20.2	Next Generation Sequencing: Listing of Detected NNRTI Resistance-associated Mutations
16.2.8.20.3	Phenotypic Data: Listing of All Available Data
16.2.8.20.4	Genotypic Interpretations with Phenotypic Data: Full Listing for HIV-1 Seroconverters

Note: Table and listing shells are provided in a separate document.

8.2. Appendix 2 – Stanford Human Immunodeficiency Virus Type 1 Drug Resistance (Database Version 8.4 Provided by Stanford HIVdb)

Stanford Mutation List NNRTI Version 8.4[9]

NNRTI

A98G, L100I, L100V, K101E, K101H, K101P, K103E, K103H, K103N, K103Q, K103R[b], K103S, K103T, V106A, V106M, V108I, E138A, E138K, E138Q, E138G, E138R, V179D, V179E, V179F, V179L, V179T, Y181C, Y181F, Y181G, Y181I, Y181S, Y181V, M184I, Y188C, Y188F, Y188H, Y188L, G190A, G190C, G190E, G190Q, G190S, G190T, G190V (H221Y, P225H, F227C, F227L, M230I, M230L, P236L, K238T, K238N, Y318F, N348I).

Other

V90I, A98S, K101N, K101Q, K101R, K103E, K103M, K103Q, K103R, V106I, V108A, I132M, I132L, V179A, V179I, V179M, Y181N, (F227I[c], F227R[c], F227V[c], Y232H, L234I[c], P236L[c]).

These 'other' mutations do not contribute to the Stanford Mutation Score for EFV, ETV, NVP or RPV, so they are not included in the NNRTI RAM summary tables. However, they are listed among the NNRTI Resistance Notes on the Stanford web page (https://hivdb.stanford.edu/dr-summary/resistance-notes/NNRTI/ last accessed on 24 March 2021), the IAS USA 2019 update of drug resistance mutations in HIV-1[10] or during in vitro passage experimentation (Module 2.7.2.4) and will be included in summaries of sequence data by codon.

[b] K103R, which by itself is polymorphic and non-scoring in the Stanford HIVdb algorithm Version 8.4, augments the V179D resistance scores for efavirenz and nevirapine from potential low-level- to intermediate resistance.

[c] These mutations are associated with resistance to doravirine.

8.3. Appendix 3 – Stanford Non-nucleoside Reverse Transcriptase Inhibitor Resistance Mutation Scores (Database Version 8.4, dated 18 July 2018)

Rule	EFV	ETR	NVP	RPV
A98G	15	10	30	15
L100I	60	30	60	60
L100V	30	10	30	15
K101E	15	15	30	45
K101H	10	10	15	10
K101P	60	60	60	60
K103H	60	0	60	0
K103N	60	0	60	0
K103S	45	0	60	0
K103T	15	0	60	0
V106A	45	0	60	0
[V106I][a]	[0]	[10]	[10]	[10]
V106M	60	0	60	0
V108I	10	0	15	0
E138A	0	10	0	15
E138G	10	10	10	15
E138K	10	10	10	45
E138Q	10	10	10	15
E138R	10	10	10	15
V179D	10	10	10	10
V179E	10	10	10	10
V179F	10	15	15	15
V179L	10	10	10	15
Y181C	30	30	60	45
Y181F	15	15	60	30
Y181G	15	15	60	30
Y181I	30	60	60	60
Y181S	15	15	60	30
Y181V	30	60	60	60
Y188C	60	0	60	0
Y188F	60	0	60	30
Y188H	30	0	60	0

Rule	EFV	ETR	NVP	RPV
Y188L	60	10	60	60
G190A	45	10	60	15
G190C	60	10	60	10
G190E	60	45	60	60
G190Q	60	45	60	45
G190S	60	10	60	15
G190T	60	10	60	10
G190V	60	10	60	10
H221Y	10	10	15	15
P225H	45	0	45	0
F227C	45	30	45	45
F227L	15	0	30	0
M230I	15	15	30	30
M230L	45	30	60	60
K238N	10	0	10	0
K238T	30	0	30	0
Y318F	10	0	30	0
N348I	0	0	15	0
K101E + Y181C	5	5	5	0
K101E + M184I	0	0	0	15
K101E + Y188L	0	5	0	0
K101E + G190A	0	5	0	0
K101E + G190S	0	5	0	0
K103R + V179D	20	0	20	15
V106A + F227L	15	0	0	0
E138K + M184I	0	0	0	15
V179F + Y181C	0	15	0	15
V179T + Y181C	0	10	0	10
Y181C + G190ACSTV	0	10	0	10
A98G + Y181C	5	5	5	

EFV = efavirenz; ETR = etravirine; NVP = nevirapine; RPV = rilpivirine
[a] Scoring is included in the current version of the Stanford HIVdb algorithm (Version 8.9-1; dated 25 October 2019).
Note: When a participant has multiple mutations, the scores for the individual mutations and for the combination of mutations have to be added in order to obtain the total score.
Example**:** A participant with mutations K101E and Y181C will have an EFV mutation score of 15 + 30 + 5 = 50.

MTN-025 VIROLOGY ADDENDUM SAP

APPENDIX 1

TABLE AND LISTING SHELLS – VERSION 1.0, 28 MAY 2021

TABLE SHELLS

Table 14.3.9.1.1
Sample Availability
Virology Population

	Statistic
HIV-1 Seroconversion Participants, N1	XX
Seroconversion Samples Available, N2 (%) (a)	xx (x.x%)
No sample n (%) (a)	xx (x.x%)
Sample not suitable n (%) (a)	xx (x.x%)
No consent n (%) (a)	xx (x.x%)
Seroconversion Samples with NGS Analysis Attempted, n (%) (b)	xx (x.x%)
HIV-1 seroconversion participants with a successful NGS genotype, N3 (%) (b)	xx (x.x%)
Sensitivity to detect RAMs present with at least:	
≤ 1% variant, n (%) (c)	xx (x.x%)
> 1% to 5% variant, n (%) (c)	xx (x.x%)
> 5% to 10% variant, n (%) (c)	xx (x.x%)
Invalid Result, N4 (%) (b, d)	xx (x.x%)
Test performed, no output (c)	xx (x.x%)
Sensitivity to detect RAMs present at:	
> 10% to 20% variant, n (%) (c,d)	xx (x.x%)
> 20% variant, n (%) (c,d)	xx (x.x%)
Seroconversion Samples with Phenotypic Susceptibility Analysis Attempted, n (%) (b)	xx (x.x%)
HIV-1 seroconversion participants with any successful Phenotypic analysis (%) (b, e)	xx (x.x%)
Invalid or No Test Result, n (%) (b)	xx (x.x%)
Failed Testing (All phenotypes), n (%) (b)	xx (x.x%)
No test (Insufficient sample volume), n (%) (b,f)	xx (x.x%)

The percentage calculation is based on the total number of participants in the denoted section.
(a) Denominator = N1. (b) Denominator = N2. (c) Denominator = N3.
(d) Due to low UMI (< 28), these are considered to provide invalid results. Mutations detected in these samples will be reported separately.
(e) In the unlikely event of one to four of the five phenotypic test results for successful analysis being missing, this should be footnoted with the affected participant identifier.
(f) Some samples may have had inadequate volume to perform both the NGS and the phenotypic tests and so analysis was performed with NGS as priority, followed by phenotype determination.

Table 14.3.9.2.1
Summary of Numbers of Participants with an Amino Acid Change from Reference per Residue in the NGS Sequenced RT Region Among HIV-1 Seroconverters using Next Generation Sequencing with Paired Population-Based Genotyping
Virology Population

Next Generation Sequencing: Number of Participants with an Amino Acid Change from Reference per Residue in the RT Region Among HIV-1 Seroconverters

	Statistic	
Sensitivity Level 1%		
Participants with a Next Generation Sequencing Assessment (1% sensitivity: UMI >/= 298) at seroconversion time point (N)	XX	
Amino Acid Change in RT (a)	**NGS n (%)**	**Paired Population-based Genotype n (%)**
Y82Y	X (XX.X%)	X (XX.X%)
Y87Y	X (XX.X%)	X (XX.X%)
Y88Y	X (XX.X%)	X (XX.X%)
Y88X + Y88Z or Y88X/Z	X (XX.X%)	X (XX.X%)
Y90Y	X (XX.X%)	X (XX.X%)
etc.		
Sensitivity Level 5%		
Participants with a Next Generation Sequencing Assessment (5% sensitivity: UMI >/= 58) at seroconversion time point (N)	XX	
Amino Acid Change in RT (a)	**NGS n (%)**	**Paired Population-based Genotype n (%)**
Y82Y	X (XX.X%)	X (XX.X%)
Y87Y	X (XX.X%)	X (XX.X%)
Y88Y	X (XX.X%)	X (XX.X%)

Y88X + Y88Z or Y88X/Z	X (XX.X%)	X (XX.X%)
Y90Y	X (XX.X%)	X (XX.X%)
etc.		

Sensitivity Level 10%

Participants with a Next Generation Sequencing Assessment (10% sensitivity: UMI >/= 28) at seroconversion time point (N) XX

Amino Acid Change in RT (a)	**NGS n (%)**	**Paired Population-based Genotype n (%)**
Y82X	X (XX.X%)	X (XX.X%)
Y87X	X (XX.X%)	X (XX.X%)
Y88X	X (XX.X%)	X (XX.X%)
Y88X + Y88Z or Y88X/Z	X (XX.X%)	X (XX.X%)
Y90X	X (XX.X%)	X (XX.X%)
etc.		

Sensitivity Level > 10%

Participants with a Next Generation Sequencing Assessment (> 10% sensitivity: UMI < 28) at seroconversion time point (N). XX

Amino Acid Change in RT (a)	**NGS n (%) (a)**	**Paired Population-based Genotype n (%)**
Y82X	X (XX.X%)	X (XX.X%)
Y87X	X (XX.X%)	X (XX.X%)
Y88X	X (XX.X%)	X (XX.X%)
Y88Z	X (XX.X%)	X (XX.X%)
Y88X + Y88Z or Y88X/Z	X (XX.X%)	X (XX.X%)
Y90X	X (XX.X%)	X (XX.X%)

etc.

N = Number of participants with data; n = number of participants with that observation.
NGS analyses should be considered exploratory.
The percentage calculation is based on the total number of participants with a next-generation sequencing assessment.
The sequenced region contains RT amino acids 80 – 150 and 152 – 212.
Participants may be counted more than once.
The reference sequence was the HXB2 sequence (NCBI accession number K03455.1, https://www.ncbi.nlm.nih.gov/nuccore/1906382 accessed 13th January 2021). Within the NGS sequencing range, this differs from the Stanford consensus B sequence only at residue 122 (consensus amino acid: lysine, 'K'; HXB2: glutamic acid, 'E').
(a) Mixtures of variants with wild-type and mutant viral species (e.g. K103N at ≤ 99% prevalence) for NGS data will be counted as the mutation being present (i.e. as 'K103N'). Similar mixtures detected using population-based genotyping (e.g. K103K/N) should be reported and counted as such. For NGS data, each mutant viral species will be counted (e.g. K103N and K103S from the same sample are counted in separate rows). Population-based genotypes having mixtures of mutations (e.g. K103N/S), will be reported as such and counted only once. Codons will be counted separately, therefore one participant with multiple mutations (e.g. K103N, E138A) will be counted more than once (i.e. twice in the example given).

[Note to programmer: Amino acids are listed in numeric order; population-based genotyping data is included as numbers are different from main analysis; residues with only null entries should be omitted; more than one variant at any residue should be reported separately; mixtures in the population-based genotypes should be counted if the mutation is present in the mixture (e.g. V108V/I would be counted with V108I in the NGS row). A separate line showing the mixture in the population-based genotype column only should then be included – mixtures will not be present by NGS. See above for Sensitivity > 10%]

Table 14.3.9.2.2
Summary of Numbers and Combinations of HIV-1 NNRTI Resistance-associated Mutations Among HIV-1 Seroconverters using Next Generation Sequencing with Paired Population-Based Genotyping
Virology Population

	Statistic	
Sensitivity Level 1% Participants with a Next Generation Sequencing Assessment (1% sensitivity: UMI >/= 298) at seroconversion time point (N)		XX
	NGS **n (%)**	**Population-based Genotype** **n (%)**
Stanford Number of NNRTI RAMs per participant (a)		
None	XX (X.X%)	XX (X.X%)
Any	X (X.X%)	X (X.X%)
One	X (X.X%)	X (X.X%)
Two	X (X.X%)	X (X.X%)
Three or more	X (X.X%)	X (X.X%)
Average	0.XX	0.XX
Stanford NNRTI resistance mutation combinations (a)		
A98G	X (X.X%)	X (X.X%)
K101E	X (X.X%)	X (X.X%)
K101E + E138A	X (X.X%)	X (X.X%)
K101E + E138A + E138K (or K101E + E138A/K (b))	X (X.X%)	X (X.X%)
etc.		

Sensitivity Level 5%
Participants with a Next Generation Sequencing Assessment (5% sensitivity: UMI >/= 58) at seroconversion time point (N) XX

	NGS n (%)	**Population-based Genotype n (%)**
Stanford Number of NNRTI RAMs per participant (a)		
None	XX (X.X%)	XX (X.X%)
Any	X (X.X%)	X (X.X%)
One	X (X.X%)	X (X.X%)
Two	X (X.X%)	X (X.X%)
Three or more	X (X.X%)	X (X.X%)
Average	0.XX	0.XX
Stanford NNRTI resistance mutation combinations (a)		
A98G	X (X.X%)	X (X.X%)
K101E	X (X.X%)	X (X.X%)
K101E + E138A	X (X.X%)	X (X.X%)
K101E + E138A + E138K (or K101E + E138A/K (b))	X (X.X%)	X (X.X%)
etc.		

Sensitivity Level 10%
Participants with a Next Generation Sequencing Assessment (10% sensitivity: UMI >/= 28) at seroconversion time point (N) XX

	NGS n (%)	**Population-based Genotype n (%)**
Stanford Number of NNRTI RAMs per participant (a)		
None	XX (X.X%)	XX (X.X%)
Any	X (X.X%)	X (X.X%)
One	X (X.X%)	X (X.X%)
Two	X (X.X%)	X (X.X%)
Three or more	X (X.X%)	X (X.X%)
Average	0.XX	0.XX
Stanford NNRTI resistance mutation combinations (a)		
A98G	X (X.X%)	X (X.X%)
K101E	X (X.X%)	X (X.X%)
K101E + E138A	X (X.X%)	X (X.X%)
K101E + E138A + E138K (or K101E + E138A/K (b))	X (X.X%)	X (X.X%)
etc. as above		

Sensitivity Level > 10%

Participants with a Next Generation Sequencing Assessment (> 10% sensitivity: UMI < 28) at seroconversion time point (N) XX

	NGS n (%)	**Population-based Genotype n (%)**
Stanford Number of NNRTI RAMs per participant (a)		
None	XX (X.X%)	XX (X.X%)
Any	X (X.X%)	X (X.X%)
One	X (X.X%)	X (X.X%)
Two	X (X.X%)	X (X.X%)
Three or more	X (X.X%)	X (X.X%)
Average	0.XX	0.XX
Stanford NNRTI RAM combinations (a)		
A98G	X (X.X%)	X (X.X%)
K101E	X (X.X%)	X (X.X%)
K101E + E138A	X (X.X%)	X (X.X%)
K101E + E138A + E138K (or K101E + E138A/K (b))	X (X.X%)	X (X.X%)
etc. as above		

N = Number of participants with data; n = number of participants with that observation; N/A = not applicable (mutations pattern was not detected); NNRTI = Non-nucleoside Reverse Transcriptase Inhibitor.
The percentage calculation is based on the total number of participants in the Virology Population and with a qualifying NGS assessment.
Participants are counted only once for each analysis (i.e. number of NNRTI RAMs per participant and number of each NNRTI RAM combination) within each sensitivity level.
The sequenced region contains RT amino acids 80 – 150 and 152 - 212. NGS analyses should be considered exploratory.
The Stanford HIV-1 Drug Resistance Database version 8.4 (dated 2017-06-16) was used.
(a) Mixtures of variants with wild-type and mutant viral minority species (e.g. K103N at ≤ 99% prevalence for NGS data or K103K/N for population-based genotyping) will be counted as the mutation being present (i.e. as 'K103N' in this example). Each sequence with a mutational pattern will be counted only once.
(b) Mixtures observed using population-based genotyping will be reported as shown in the example 'E138A/K', whereas these will be reported separately as 'E138A' and 'E138K' using NGS.

[Note to programmer: Amino acids are listed in numeric order; population-based genotyping included as numbers might be different from main analysis. To simplify the analysis of population-based genotype mixtures, include the count if the mixture includes a variant from the NGS analysis. Also, if there is more than one variant detected by NGS, these should be included in the listed row. The example: K101E + E138A + E138K + Y181C might be represented in the population-based genotype as K101E + E138A/K + Y181C the E138A + E138K would count as one mutation, but subsequently broken down by sequence. The example shows all possible population-based genotypes, only ones that apply should be included.]

Table 14.3.9.3.1
Summary of Phenotypic Susceptibility to Dapivirine and Other NNRTIs by Population-based Genotype
Virology Population

All Viruses	**Dapivirine**	**Efavirenz**	**Etravirine**	**Nevirapine**	**Rilpivirine**
N	XX	XX	XX	XX	XX
IC_{50} (nM)					
Geometric mean (SD)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)
(25th/median/75th)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)
(Min,max)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)
Fold change (a)					
Geometric mean (SD)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)
(25th/median/75th)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)
(Min,max)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)
Wild-type viruses (b)					
N	XX	XX	XX	XX	XX
IC_{50} (nM)					
Geometric mean (SD)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)
(25th/median/75th)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)
(Min,max)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)
Fold change (a)					
Geometric mean (SD)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)
(25th/median/75th)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)
(Min,max)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)

Viruses with any NNRTI RAM					
N	XX	XX	XX	XX	XX
IC_{50} (nM)					
Geometric mean (SD)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)
(25th/median/75th)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)
(Min,max)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)
Fold change (a)					
Geometric mean (SD)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)
(25th/median/75th)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)
(Min,max)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)
Viruses with one NNRTI RAM					
N	XX	XX	XX	XX	XX
IC_{50} (nM)					
Geometric mean (SD)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)
(25th/median/75th)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)
(Min,max)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)
Fold change (a)					
Geometric mean (SD)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)
(25th/median/75th)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)
(Min,max)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)

Viruses with two NNRTI RAMs					
N	XX	XX	XX	XX	XX
IC_{50} (nM)					
Geometric mean (SD)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)
(25th/median/75th)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)
(Min,max)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)
Fold change (a)					
Geometric mean (SD)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)
(25th/median/75th)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)
(Min,max)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)
Viruses with three or more NNRTI RAMs					
N	XX	XX	XX	XX	XX
IC_{50} (nM)					
Geometric mean (SD)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)
(25th/median/75th)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)
(Min,max)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)
Fold change (a)					
Geometric mean (SD)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)	x.xx (x.xxx)
(25th/median/75th)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)	(x.xx/x.xx/x.xx)
(Min,max)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)	(x.xx/xx.xx)

Viruses with A98G

etc.

Viruses with K101E

etc.

Viruses with K101E + E138A

etc.

(a) Fold change = $IC_{50}/IC_{50Control}$.
(b) Mutations are included from population-based genotyping results (minority species mutations are less likely to influence phenotypes).

Table 14.3.9.4.1
Summary of Number of Participants with an Amino Acid Change from Reference per Residue in the RT Region Among HIV-1 Seroconversions using Population-Based Genotyping (All Seroconversion ± 14-Days Data)
Virology Population

	Population-based Genotype n (%)
Participants with a population-based genotyping assessment at seroconversion time point (N)	XX
Stanford Number of NNRTI RAMs per participant (a)	
None	XX (X.X%)
Any	X (X.X%)
One	X (X.X%)
Two	X (X.X%)
Three or more	X (X.X%)
Average	0.XX
Stanford NNRTI resistance mutation combinations (b)	
A98G	X (X.X%)
K101E	X (X.X%)
K101E + E138A	X (X.X%)
K101E + E138A/K + Y181C	X (X.X%)
etc.	

N = Number of participants with data; n = number of participants with that observation; NNRTI = Non-nucleoside Reverse Transcriptase Inhibitor.
The percentage calculation is based on the total number of participants with a successful population-based genotyping assessment at seroconversion.
The Stanford HIV-1 Drug Resistance Database version 8.4 (dated 2017-06-16) was used.
(a) Instances of mixtures among the population-based genotypes that include one or more NNRTI RAM, are counted as if the mutation with the greatest drug resistance score was present.

LISTING SHELLS

Listing	Title
16.2.8.20	**Next Generation Sequencing and Phenotype Susceptibility Data**
16.2.8.20.1	Next Generation Sequencing: Listing of All Available Data
16.2.8.20.2	Next Generation Sequencing: Listing of Detected NNRTI Resistance-associated Mutations
16.2.8.20.3	Phenotypic Data: Listing of All Available Data
16.2.8.20.4	Genotypic Interpretations with Phenotypic Data: Full Listing for HIV-1 Seroconverters

Note: Population-based genotyping data are listed in the MTN-025 CSR Tables, Listings and Figures.

Listing 16.2.8.20.1
Next Generation Sequencing: Listing of All Available Data
Exposed Primary Population

PID (a)	Sample Date	Days (b)	Days (c)	Visit Day (d)	Log_{10} HIV-1 RNA (copies/mL)	Number UMI with Consensus	Sample Sensitivity (%)	Valid Result	Amino acid (% UMI) Change from Reference (e)
XXX-XXXXXX	DDMMMYYYY	XX	XX	XXX	X.XXX	XXXX	X	Yes/No	YxxX (xx), YxxX (xx), YxxxX (xx),….
etc.									

(a) PIDs infected on enrolment indicated with 'α'; PIDs with seroconversion off DVR-004 use indicated with 'β'.

(b) Days since seroconversion (negative values indicate evaluation prior to seroconversion).

(c) Days since first HIV-1 RNA detection.

(d) Visits indicated with a * are not within 14 days of the seroconversion time point.

(e) Reference sequence: HXB2 (accession number K03455-1).

The sequenced region contains RT amino acids 81 – 150, 152 – 212. NGS analyses are considered exploratory.

[Notes to programmer: Amino acids are listed in numeric order (Note e); choose appropriate markers for different populations (Note a).]

Listing 16.2.8.20.2
Next Generation Sequencing: Listing of Detected NNRTI Resistance-associated Mutations
Exposed Primary Population

PID (a)	Sample Date	Days (b)	Days (c)	Visit Day	Visit (d)	Number UMI with Consensus	Sample Sensitivity (%)	Number of Mutations	NNRTI RAMs (e)
XXX-XXXXXX	DDMMMYYYY	XX	XX	XXX	Month XX/ Week XX	XXXX	X	X	YxxZ (xx), YxxZ (xx),….
XXX-XXXXXX	DDMMMYYYY	XX	XX	XXX	Month XX/ Week XX	XXXX	X	X	None
XXX-XXXXXX	DDMMMYYYY	XX	XX	XXX	Month XX/ Week XX	XXXX	X	X	No Test

etc.

Table includes PIDS only if NNRTI RAMs were detected in NGS analysis.

(a) PIDs infected on enrolment indicated with 'α'; PIDs with seroconversion off DVR-004 use indicated with 'β'.

(b) Days since seroconversion (negative values indicate evaluation prior to seroconversion).

(c) Days since first HIV-1 RNA detection.

(d) Visits indicated with a * are not within 14 days of the seroconversion time point.

(e) Mutations reported according to Stanford HIVdb algorithm version 8.4 (see SAP).

The sequenced region contains RT amino acids 81 – 150, 152 – 212. NGS analyses should be considered exploratory.

[Notes to programmer: Amino acids are listed in numeric order (Note e); choose appropriate markers for different populations (Note a).]

Listing 16.2.8.20.3
Phenotypic Data: Listing of All Available Data
Exposed Primary Population

				DPV		EFV		ETR		NVP		RPV	
PID	Sample Date	NNRTI RAMs	Result ID	IC_{50} (nM)	FC (a)	IC_{50} (nM)	FC (a)	IC_{50} (nM)	FC (a)	IC_{50} (nM)	FC (a)	IC_{50} (nM)	FC (a)
XXX-XXXXXX	DDMMMYYYY	None	1	x.xx (x.xx)		x.xx (x.xx)		x.xx (x.xx)		x.xx (x.xx)		x.xx (x.xx)	
			2	x.xx (x.xx)									
			3	x.xx (x.xx)									
			Avg	x.xx (x.xx)	x.x	x.xx (x.xx)	x.x	x.xx (x.xx)	x.x	x.xx (x.xx)	x.x	x.xx (x.xx)	x.x

etc.

DPV = Dapivirine; EFV = Efavirenz; ETR = Etravirine; FC = Fold change; IC_{50} = 50% Inhibitory concentration; NNRTI = Non-nucleoside reverse transcriptase inhibitor; NVP = Nevirapine; PID = Participant identifier; RAM = Resistance-associated mutation; RPV = Rilpivirine
(a) FC = $IC_{50}/IC_{50Control}$ (Control IC_{50} [nM] range: DPV: n = X: X.XX - X.XX; EFV: n = X: X.XX - X.XX ; ETR: n = X: X.XX - X.XX; NVP: n = X: X.XX - X.XX; RPV: n = X: X.XX - X.XX). n: number of runs to complete the analysis.
(b) IC_{50} sample and (IC_{50} reference virus) are given.

[Note to programmer: Order by PID and then by NNRTI RAMs with 'None' first, then mutations in numeric order. Note the reference virus will be different from that in MTN-020 - a single lab-strain has been used per run.]

Listing 16.2.8.20.4
Genotypic Interpretations with Phenotypic Data: Full Listing for HIV-1 Seroconverters
Exposed Primary Population

		NNRTI RAMs			NNRTI Genotypic Susceptibility (Population-based sequencing)	Phenotype	
PID	Sample Date	Population	NGS	NNRTI	(a)	IC_{50} (nM)	FC (b)
XXX-XXXXXX	DDMMMYYYY	None	None	Dapivirine	N/A	X.XX	X.X
				Efavirenz	XX (YYY)	X.XX	X.X
				Etravirine	XX (YYY)	X.XX	X.X
				Nevirapine	XX (YYY)	X.XX	X.X
				Rilpivirine	XX (YYY)	X.XX	X.X
XXX-XXXXXX	DDMMMYYYY	YXXY	YXXY	Dapivirine	N/A	X.XX	X.X
				Efavirenz	XX (YYY)	X.XX	X.X
				Etravirine	XX (YYY)	X.XX	X.X
				Nevirapine	XX (YYY)	X.XX	X.X
				Rilpivirine	XX (YYY)	X.XX	X.X

etc.

HLR = High-level resistant; Interm = Intermediate resistant; LLR = Low-level resistant; N/A = Not applicable; NNRTI = Non-nucleoside Reverse Transcriptase Inhibitor; PLLR = Potential low-level resistant; RAM = Resistance-associated mutation; Sus = Susceptibility.

(a) Stanford HIVdb algorithm version 8.4 drug resistance score (interpretation: Sus, PLLR, LLR, Interm, HLR).
(b) FC = $IC_{50}/IC_{50CONTROL}$ (Control IC_{50} [nM] range: DPV: n = X: X.XX - X.XX; EFV: n = X: X.XX - X.XX ; ETR: n = X: X.XX - X.XX; NVP: n = X: X.XX - X.XX; RPV: n = X: X.XX - X.XX)

[Note to programmer: Order by PID then by NNRTI RAMs with 'None' first, then mutations in numeric order (as per PhenoList).]