Understanding Adherence to and HIV-1 Protection from a Microbicidal Vaginal Ring

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Abstract

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Topical vaginal microbicides and pre-exposure prophylaxis (PrEP) in the form of oral pills have been evaluated in efficacy trials for the prevention of HIV-1 infection over the past two decades with mixed results. In intention-to-treat analyses, some studies of PrEP agents, such as Truvada®, have shown positive efficacy results for reducing rates of HIV-1 infection in populations with high levels of adherence, while other studies even with the same agents and mostly among women, have not. However, in post-hoc analyses accounting for adherence to product use, efficacy estimates increased when adjusting for adherence in these same studies. The International Partnership for Microbicides developed a vaginal ring containing a PrEP agent called dapivirine to be replaced on a monthly basis, as a means to address the challenges women face adhering to PrEP agents requiring more frequent use.

The goal of this dissertation was to further our understanding of the adherence to and HIV-1 protection from the dapivirine vaginal ring when used in a clinical trial setting among African women at high risk for HIV-1 acquisition. To this end, we utilized data from the MTN-020/ASPIRE

study in which 2,629 African women participated in a randomized, placebo-controlled phase III trial of the dapivirine vaginal ring for the effectiveness of HIV-1 prevention conducted by the Microbicides Trials Network (MTN). The specific aims of this dissertation were 1) to assess the feasibility and effectiveness of real-time adherence monitoring strategies utilized within the MTN-020/ASPIRE study; 2) to evaluate the efficacy of a vaginal ring containing dapivirine compared to a placebo vaginal ring accounting for varying levels of adherence to product use; and 3) to identify demographic, sexually transmitted disease, and risk behavior correlates to adherence to inform future implementation of the dapivirine vaginal ring. We addressed Aim 1 by first describing the methods for implementation of the real-time adherence monitoring strategy. Using generalized estimating equations, we then assessed its effectiveness by evaluating whether percentages of blood plasma samples with dapivirine >95 pg/mL in active arm participants increased over calendar time. For the second aim, we conducted three analyses utilizing methods that adjust for adherence to ring use in different ways, specifically, per-protocol, principal stratification and marginal structural models. Four cut points for adherence were defined as follows: >95 pg/mL and >200 pg/mL of blood plasma, and ≥0.9 mg and >4.0 mg of drug released from the rings over a 28-day period of expected ring use. The per-protocol analysis aimed to emulate accrual under perfect protocol adherence, censoring participants at the last negative HIV-1 test before detection of pregnancy or the first occurrence of three or more non-compliant events in a 12-month period. In the principal stratification analysis, we first constructed logistic regression models to predict adherence defined as ≥0.9 mg and >4.0 mg of drug released from the rings at 6 months, and 12 months for sensitivity analyses, using baseline characteristics from only dapivirine arm participants' data. Second, we applied this model to the entire cohort to obtain predicted probabilities of adherence, and last we constructed a Cox proportional hazards model with treatment arm, predicted probabilities of

adherence and the interaction between the two variables. The marginal structural models analysis was based on the potential outcomes framework for causal inference and utilized inverse-probability-of-censoring weights (IPCWs) and an inverse-probability-of-treatment weight (IPTW) to create a pseudo-population free of confounding and selection bias as a result of informative censoring. We constructed pooled logistic regression models with restricted cubic splines to represent time on study, to develop two IPCWs, the first for the outcome of first pregnancy, and the second for the outcome of informative loss to follow-up. An IPTW model was also developed based on whether the participant was adherent using the ≥0.9 mg cutoff of drug released from the ring. A final pooled logistic regression model was constructed with a re-weighted population using a final stabilized weight calculated by multiplying together the two IPCWs and the IPTW. The last aim of this dissertation involved the discovery of correlates to adherence as defined by drug levels in plasma and estimated drug release levels from the vaginal ring. A multivariable generalized estimating equation model was constructed for each of the four adherence outcomes.

Among a total of 2,629 women enrolled in MTN-020/ASPIRE, median age was 26 years (IQR: 22-31), and median follow-up time was 1.6 years (IQR: 1.1-2.3). In Aim 1, we found an upward linear trend of adherence over calendar time, from 63% during Quarter 1 2013 to 84% during Quarter 1 2015 ($p < 0.0001$). In Aim 2, in per-protocol analyses, we found HIV-1 efficacy estimates of 30.8% (3.6%, 50.3%) ($p = 0.03$), whereas from principal stratification analyses among the highest adherers HIV-1 efficacy estimates using 6 month data with the ≥0.9 mg cutoff was 53.6% (16.5%, 74.3%) ($p = 0.01$). Marginal structural models produced HIV-1 efficacy estimates ranging from 48.8% (21.8%, 66.4%) ($p = 0.0019$) to 56.5% (32.8%, 71.9%) ($p = 0.0002$). From the correlates of adherence analysis in Aim 3, we found that time on study, calendar time, primary partner

knowledge that the participant was taking part in the study, and use of long-acting contraceptive methods were associated with ring adherence whereas younger age, ring worries, condom use during sex, episodes of menstrual bleeding and vaginal washing were associated with ring non-adherence.

We found in Aim 1 that ongoing drug level testing as a marker of adherence in MTN-020/ASPIRE demonstrated the feasibility of real-time adherence monitoring while maintaining study blinding at the level of participants, sites, and study leadership. We found in Aim 2 that per-protocol analyses only modestly improved on intention-to-treat efficacy estimates, whereas estimates increased for strata of more highly adherent participants, and when adjusting for time-varying confounding and selection bias due to informative censoring. Finally, in Aim 3 we found several important predictors of adherence that may prove to be useful for recruitment into future clinical studies and for dapivirine ring implementation efforts. Therefore, all hypotheses evaluated in this dissertation were shown to be true.

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DEDICATION

To my mother, Shirley (Van Tassell) Husnik and my late father, Franklin Husnik. I would like to thank them both from the bottom of my heart for all they have given me throughout my life and for always supporting me as I pursued my life's dreams.

Chapter 1. INTRODUCTION

1.1 BACKGROUND

The goal of this research was to examine in depth the use of and protection from a microbicidal vaginal ring for human immunodeficiency virus type 1 (HIV-1) prevention in women. Women account for more than half of the 37.9 million people living with HIV-1, and women in Africa face high HIV-1 risk.[1] Topical vaginal microbicides and pre-exposure prophylaxis (PrEP) in the form of oral pills have been evaluated in efficacy trials for the prevention of HIV-1 infection over the past two decades with mixed results. Some studies of PrEP agents such as Truvada® [emtricitabine (FTC)/tenofovir disoproxil fumarate (TDF)] in intention-to-treat analyses have shown positive efficacy results for reducing rates of HIV-1 infection in populations with high levels of adherence[2-4], while other studies with the same agents with lower levels of adherence, have not.[5-7] However, in post-hoc analyses accounting for adherence to product use, efficacy estimates trended upward in these same studies. The International Partnership for Microbicides developed a vaginal ring containing a PrEP agent called dapivirine for women to use on a monthly basis, as a means to address issues of adherence.[8] More than twenty phase I/II clinical studies to date have assessed the safety and tolerability of dapivirine in vaginal rings, gels and oral formulations with promising safety and tolerability profiles.[9] Data used for this dissertation were from a clinical trial sponsored by the Microbicides Trials Network (MTN) entitled MTN-020/ASPIRE: A Multi-Center, Randomized, Double-Blind, Placebo-Controlled Phase 3 Safety and Effectiveness Trial of a Vaginal Matrix Ring Containing Dapivirine for the Prevention of HIV-1 Infection in Women. Adherence to and HIV-1 prevention from a vaginal ring within a large-scale efficacy study was assessed in MTN-020/ASPIRE, and analyses beyond the primary intention-to-treat efficacy

findings would be important for fully understanding the relationship between adherence and HIV-1 protection.

1.2 Specific Aims, Rationale and Hypotheses

The specific aims, rationale for the aim and our hypotheses for this dissertation are as follows:

Aim 1: To assess the feasibility and effectiveness of real-time adherence monitoring strategies utilized within MTN-020/ASPIRE.

Rationale: A real-time adherence monitoring strategy using an objective marker of product use while preserving the blinding of the study was conducted during MTN-020/ASPIRE. This approach is novel for large-scale efficacy studies for HIV-1 prevention.

Hypothesis: Shipping, testing and analysis of dapivirine levels in plasma will be completed and communication of the results made to the study sites on at least a quarterly basis from commencement of the real-time monitoring system. In addition, improved adherence to product use as measured by a statistically significant increasing trend of dapivirine levels at the >95 pg/mL levels over time will be observed.

Aim 2: To evaluate the efficacy of a vaginal ring containing dapivirine compared to a placebo vaginal ring accounting for varying levels of adherence to product use in MTN-020/ASPIRE.

Rationale: Intention-to-treat analyses, in the presence of imperfect adherence, are limited to assessing a combined effect of adherers and non-adherers. However, alternative analytical

approaches have been proposed to provide unbiased estimates of efficacy in the context of incomplete follow-up and/or non-compliance to product use.

Hypothesis: The estimated relative hazard of HIV-1 infection associated with dapivirine will be lowest among women who are more-fully adherent compared to those who are less-fully adherent.

Aim 3: To identify demographic and self-reported risk behaviors that are correlates to adherence to the use of the microbicidal vaginal ring.

Rationale: Identifying correlates to adherence to the use of a microbicidal vaginal ring may be useful for clinicians if a microbicidal ring were found to be effective. Likewise, identification of persons at risk for low adherence prior to enrollment in a clinical study could be used to improve efficiency in recruitment of clinical trial participants.

Hypothesis: In exploratory analyses, multiple correlates to adherence will be identified that are statistically significant at the $p < 0.05$ level.

1.3 SIGNIFICANCE

This dissertation addresses the critical issue of adherence to product use that underlies the observed effectiveness of microbicidal interventions for the prevention of HIV-1. Measuring, accounting for, and improving adherence are key analytic and implementation concepts essential in the investigation of antiretroviral prevention strategies. Real-time adherence monitoring is rarely used in clinical trial settings and the reliance on behavioral measures as indicators for adherence often

fall short. For example, in the MTN-003/VOICE study, a five-arm, randomized efficacy trial conducted in 5,029 women from 15 clinical research sites in Uganda, South Africa and Zimbabwe and conducted from September 2009 to August 2012, Truvada® or TDF versus a placebo pill, and 1% tenofovir gel versus a placebo gel were evaluated for HIV-1 efficacy.[5] The trial showed null findings in spite of reported adherence levels of ≥86% for each of daily use of pill and applicator counts, face-to-face questioning, and audio computer-assisted self-interviews. However, and surprisingly, adherence to product use as measured through plasma levels of tenofovir in a sub-cohort of 647 randomly-chosen participants from the active-arms was very low and at odds with the behavioral measures: 25% in the 1% tenofovir gel arm, 29% in the Truvada® arm and 30% in the TDF arm. The divergence between self-reported behavioral measures of adherence and biological measures seen in the MTN-003/VOICE study underlies the importance of implementing and evaluating a real-time adherence monitoring system in future efficacy trials of microbicidal agents that can utilize both self-reported behavioral measures and also biological measures. Additional PrEP studies that have taken into effect adherence levels are shown in Appendix I, and a plot of intention-to-treat efficacy results compared to adherence is shown in Appendix II. Results of our analyses will help to inform the utility of implementing adherence monitoring in real-time for future large-scale trials.

Post-hoc analyses whereby participants are grouped based on post-randomization adherence levels have been useful in prior trials but may be subject to selection bias. Moreover, adherence is typically framed, measured and defined as an effect modifier of treatment efficacy within clinical trials.[10-11] In fact, several studies to date have been aimed at measuring efficacy of microbicidal gels, and the impact of adherence, as an effect modifier. These studies examined efficacy for gel products such as Col 1492 Nonoxynol-9, BufferGel and 0.5% PRO2000 gel, Carraguard, Cellulose

Sulfate, MDP 301 PRO2000/5 and Tenofovir.[12-19] For example, topically-applied microbicidal products have been tested in clinical trials over the past two decades and one study in humans to date (CAPRISA 004) has provided evidence that an antiretroviral agent, 1% tenofovir, in a gel applied pre- and post-coitally compared to a placebo is effective in preventing HIV-1 transmission.[12] Investigators observed an overall effectiveness based on intention-to-treat analyses of 39% (95% CI: 6%, 60%; p=0.017). In analyses among participants whose gel adherence was >80%, the effectiveness was 54% (95% CI: 4%, 80%; p=0.025), in participants whose gel adherence was 50% to 80%, the effectiveness was 38% (95% CI: -67%, 77%; p=0.343), and among participants whose gel adherence was <50%, the effectiveness was reduced further to 28% (95% CI: -40%, 64%; p=0.303). Although in the latter two adherence groups the results were not statistically significant, efficacy estimates differed according to levels of adherence highlighting its importance within a clinical trial setting. Moreover, in oral PrEP trials, daily regimens consisting of 300 mg of TDF alone or in combination with 200 mg of FTC have been found to reduce the risk of HIV-1 acquisition by 50% or more among those with high adherence to product use; this was demonstrated in men who have sex with men (with up to 100% reduction in HIV-1 transmission among the most adherent men based on blood levels), heterosexuals and injection-drug users.[2-4,20] Thus, it's clear that in these post-hoc analyses product efficacy was substantially greater where adherence was treated as an effect modifier than in the intention-to-treat comparisons. However, the application of novel analytical approaches has been proposed to account for time-varying confounding and selection bias; that is, those who self-select to adhere to treatment regimens may differ from those who do not with respect to baseline risk for HIV-1 infection. We utilized per-protocol analyses accounting for time off product due to missed visits, as well as causal inference methods of principal stratification approach with potential outcomes[21], and inverse-probability of censoring and treatment weighting in marginal structural models.[22-25]

The per-protocol models aimed to emulate accrual under perfect protocol adherence, censoring participants at the last negative HIV-1 test before detection of pregnancy or the first occurrence of three or more non-compliant events in a 12 month period which include the following: a missed visit, participants reporting the ring being out for more than 12 hours, and participants failing to return product to the clinical site. We used the latter two approaches to apply the potential outcomes framework with instrumental variables model to the MTN-020/ASPIRE dataset. We aimed to obtain unbiased estimates of the average causal effect among high adherers for the principal stratification approach. For marginal structural models we aimed to build a pseudo-population by re-weighting participants' data to account for potential confounding and selection bias due to informative censoring to obtain unbiased estimates of the average causal effect of the dapivirine vaginal ring compared to a placebo on the risk of HIV-1 infection.

Prior studies have developed models identifying predictors of high and low adherence.[26-27] Likewise, we believe there is utility in knowing the correlates for high and low adherence with respect to post-hoc analyses and understanding populations to target for future research studies. To this end, we developed generalized estimating equation models utilizing both baseline and longitudinal measures to identify correlates to four biological measures for ring adherence, representing recent and cumulative use. These measures are based on two levels of quarterly dapivirine blood plasma (>95 pg/mL and >200 pg/mL), and two levels of dapivirine released from returned rings (≥0.9 mg and >4.0 mg), respectively. The identification of correlates to ring use, utilizing these various measurements of adherence, in turn, may lead to patient counseling by clinicians to improve uptake if a microbicidal ring such as the dapivirine ring were licensed for use whereby the acceptability and adherence to product use will be of great importance to scale-up efforts. Likewise, identification of persons who may have low adherence based on correlates

found in these analyses prior to enrollment in a clinical study may be used to improve efficiency in recruitment of clinical trial participants. Finally, these findings inform future work to understand predictors for product adherence of a vaginal ring containing a PrEP agent such as dapivirine, or potentially other user-defined products.

1.4 BACKGROUND OF THE MTN-020/ASPIRE STUDY

MTN-020/ASPIRE is entitled A Multi-Center, Randomized, Double-Blind, Placebo-Controlled Phase III Safety and Effectiveness Trial of a Vaginal Matrix Ring Containing Dapivirine for the Prevention of HIV-1 Infection in Women. ASPIRE is a multi-site, two-arm, double-blind, randomized (1:1), placebo-controlled phase III clinical trial to evaluate the safety and effectiveness of dapivirine (25 mg) in a silicone elastomer vaginal matrix ring, inserted once every four weeks for the prevention of HIV-1 infection in healthy, sexually active HIV-1-uninfected women, when compared to a placebo vaginal ring. The study enrolled women from fifteen study sites in four African countries: Malawi (Blantyre, Lilongwe), Uganda (Kampala), South Africa [Cape Town, Durban (7 sites), Johannesburg] and Zimbabwe [Chitungwiza (2 sites), Harare].

The primary objectives were as follows: 1) To determine the effectiveness of dapivirine administered in a silicone elastomer vaginal matrix ring, when inserted once every four weeks, in preventing HIV-1 infection among healthy sexually active HIV-1-uninfected women; and 2) to assess the safety of dapivirine administered in a silicone elastomer vaginal matrix ring, when inserted once every four weeks over the investigational product use period. The secondary objectives were as follows: 1) To evaluate the acceptability of the study vaginal ring (dapivirine or placebo) in HIV-1-uninfected women, when inserted once every 4 weeks over the

investigational product use period; 2) to evaluate the adherence to the study vaginal ring (dapivirine or placebo) in HIV-1-uninfected women, when inserted once every 4 weeks over the investigational product use period; 3) to assess the frequency of HIV-1 drug resistance in women who acquire HIV-1 infection while using the investigational product; and 4) to evaluate the relationship between drug concentration and HIV-1 seroconversion.

The first study site was activated on July 19, 2012, the first enrollment occurred on August 21, 2012 and last enrollment occurred on June 12, 2014. A total of 5,516 participants were screened and 2,629 were enrolled. Follow-up was completed in June 2015 when 168 HIV-1 infection endpoints were observed. Participants had up to 33 months of follow-up visits.

1.5 Research Design and Study population

The design of the studies in this dissertation are prospective cohort studies among sexually active HIV-1-uninfected African women enrolled in the MTN-020/ASPIRE study. See Appendix III for further details regarding the inclusion/exclusion criteria.

1.6 Data Collection/Data Management/Data Quality

Assessments were made at monthly, quarterly, and semi-annual visits via case report forms (CRFs) and/or audio computer assisted self-interview according to the schedule of study visits and evaluations seen in Appendix IV. Evaluations specific to the studies in this dissertation are highlighted in yellow. Regarding ring dispensation and removal, at monthly visits, participants were asked (or assisted) to remove their previous month's vaginal ring which was then replaced

by a new ring before the participant completed the visit. All follow-up visits consisted of data collection for behavioral, clinical, laboratory, pelvic exams and dispensation/collection of study product. Site staff faxed CRFs to the Statistical Center for HIV/AIDS Research and Prevention (SCHARP) at the Fred Hutchinson Cancer Research Center in Seattle, Washington. Data from CRFs were received at SCHARP via the DataFax (DF) software system and were verified twice by data operations specialists. Laboratory data were received at SCHARP from the MTN network laboratory via the SCHARP ATLAS Science Portal. Data quality control checks were programmed in the DF system and reports were sent to clinical sites on a monthly basis to resolve data issues.

1.7 HIV-1 Status Determination

HIV-1 status was defined via the testing algorithm shown in Appendix V. Specifically, two HIV-1 rapid tests were conducted at the clinical sites at each monthly visit. If at least one HIV-1 rapid test was positive, then Western Blot confirmatory testing was conducted at the clinical site. In addition, blood plasma was collected for HIV-1 confirmatory testing by the MTN network laboratory. In the case of non-confirmable results via network laboratory testing, an endpoint adjudication committee reviewed relevant laboratory results and provided the final HIV-1 status.

1.8 Dapivirine Levels in Blood Plasma

Blood plasma was collected and stored at quarterly and semi-annual visits. Dapivirine concentrations in plasma were quantified via validated ultra-performance liquid chromatographic-tandem mass spectrometric (LC-MS/MS) methods.[28-30] This method has an analytical measurement range for dapivirine in plasma from the lower limits of quantification of 20 to 10,000

pg/mL. For the purposes of monitoring adherence during the trial, a cut point of >95 pg/mL was chosen in order to distinguish participants who had the ring in place for at least several hours prior to the visit. A prior study conducted by Nel et al examined the pharmacological properties of the same dapivirine matrix vaginal ring used in the MTN-020/ASPIRE study and determined that the mean concentrations of dapivirine in blood plasma increased in the first four hours of use to a T_{max} of 8 hours where the least squares mean for the maximum concentration (C_{max}) was 1194 pg/mL.[31] Moreover, sustained use of the dapivirine vaginal ring through 28 days showed mean plasma concentrations of 160 pg/mL, and once the vaginal ring was removed, a steady decrease per day over the next five days to a level of 35 pg/mL at day 33. The terminal half-life after removal on day 28 was also estimated to be 64 hours. These measures are used in Aim 1 and Aim 3.

1.9 Ring Adherence Measures

Participants returned rings to the clinical sites on a monthly basis. After the first year of the study, the active arm participants' rings were sent to a central laboratory for testing. Residual drug levels for each ring were documented and sent to SCHARP for data processing. Using residual drug levels, the amount of dapivirine released from returned rings was calculated based on manufacturing ring load levels and an expected 28-day period of monthly use. Using these estimates, two cut points were defined for recent use (≥0.9 mg of drug released in a 28-day monthly period) and continuous use (>4.0 mg of drug released in a 28-day monthly period). These measures are used in Aim 2 and Aim 3.

1.10 BEHAVIORAL MEASURES

Covariates used in Aim 2 and Aim 3 are based on self-reported behavioral data collected on the Behavioral Assessment CRF at each quarterly visit. We constructed indicators, categorical variables and/or continuous variables as appropriate for the following: whether the participant was worried about having a vaginal ring inside of her every day for at least a year, whether the participant reported having a primary partner in the past three months, HIV-1 status of primary partner, whether primary sex partner came to clinic with participant in the past month, number of other sex partners in past three months, number of vaginal sex acts in last seven days, and number of vaginal sex acts protected by a male or female condom in the last seven days. In addition, we utilized data collected from the Demographics, Family Planning CRFs and Social Impact Log CRFs for the following: age, marital status, highest level of education, study site, and type of contraceptive use (intrauterine device, oral contraceptives, injectable contraceptives, implants, condoms, and sterilization).

Chapter 2. IMPLEMENTATION OF A NOVEL ADHERENCE MONITORING STRATEGY IN A PHASE III, BLINDED, PLACEBO-CONTROLLED, HIV-1 PREVENTION CLINICAL TRIAL

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2.1 ABSTRACT

Background: Placebo-controlled HIV-1 prevention trials of pre-exposure prophylaxis (PrEP) have not generally used concurrent measurement of adherence due to the potential risk of unblinding. However, several PrEP trials for HIV-1 prevention among women failed to show effectiveness due to low product adherence. Evaluation of product adherence objectively during a study provides the opportunity for strengthening adherence activities at sites having low adherence.

Methods: During MTN-020/ASPIRE, a phase III, placebo-controlled trial of the dapivirine intravaginal ring, we implemented an adherence monitoring system. Monitoring began in Quarter 1 (Q1) 2013 and continued through the conclusion of the trial. Blood plasma was collected quarterly and tested for dapivirine concentrations while maintaining blinding among study team members involved in participant management. Dapivirine concentrations >95 pg/mL, reflecting >8 hours of continuous use, were assessed as signaling product use. Study leadership monitored results on a monthly basis and provided feedback to site investigators. Experiences were shared across sites to motivate staff and counsel participants to strive toward higher adherence levels.

Results: An upward trend in adherence was observed ($p < 0.0001$); the proportion of samples from subjects in the active arm with dapivirine >95 pg/mL increased from 63% in Q1 2013 to 84% by Q1 2015.

Conclusions: Ongoing drug level testing as a marker of adherence in MTN-020/ASPIRE demonstrates the feasibility of real-time adherence monitoring while maintaining study blinding

at the level of participants, sites, and study leadership. This approach is novel for large-scale effectiveness studies for HIV-1 prevention.

Keywords: HIV-1, adherence, dapivirine, women, pre-exposure prophylaxis, microbicide, intravaginal ring

2.2 INTRODUCTION

In Africa and worldwide, women account for more than half of all people living with HIV-1, and young women in Africa can often face strikingly high HIV-1 risk.[32] Development of effective prevention options for women is thus a global health priority. Over the past decade, a number of studies have evaluated novel antiretroviral-based prevention strategies, including topical vaginal microbicides and oral pre-exposure prophylaxis (PrEP), for HIV-1 prevention in young African women. In three placebo-controlled, pivotal studies of oral tablet and vaginal gel formulations of the antiretroviral agent tenofovir among young African women, adherence to the investigational products was extremely low and HIV-1 protection was not seen,[5-7] in contrast, these products demonstrated efficacy for HIV-1 protection in other studies where adherence was higher.[2-4,12,20,33] Although all of these PrEP studies used multiple methods to evaluate product adherence while the trials were ongoing, including self-reported use and counts of unused study product returns, those approaches were subsequently found to have substantially over-estimated adherence, when compared with measurement of the active investigational medication in plasma or cervicovaginal fluids.[5-7] The surprising blood and cervicovaginal testing results were not consistent with pre-study expectations that the target population had a high willingness to adhere to product use.[34]

The discovery of very low adherence on biologic samples tested after completion of tenofovir-based prevention trials among women was a surprise to the HIV prevention field,[35] as an implicit assumption of randomized trials has been that study participants who regularly attend monthly study visits, have pregnancy and HIV-1 testing, and receive risk reduction and product adherence counseling will also use investigational study products. Moreover, no difference was seen in women's tendency to accurately report non-adherence at the study termination visit and at routine

follow-up visits underscoring that women in resource limited settings often join studies for the benefits of study participation and fear that by revealing product non-use their study participation could be jeopardized.[36] Strategies to evaluate adherence objectively during the study provides an opportunity for study investigators to strengthen adherence counseling, adjust study enrollment at some sites, or even terminate a study if low adherence suggests futility. However, blinded, placebo-controlled HIV-1 prevention clinical trials have not generally implemented real-time monitoring of adherence using measures of the active investigational medication due to the costs and the potential risk of unblinding of study leadership, clinical staff, and participants to randomization arm assignments.

An intravaginal ring containing the antiretroviral agent dapivirine was developed as a novel topical microbicide and tested in phase I-III trials,[31,37-39] including two recently-completed phase III trials, MTN-020/ASPIRE[40] and IPM 027/The Ring Study.[41] Within the MTN-020/ASPIRE trial, we implemented an adherence monitoring system, concurrent with the conduct of the trial, using measurement of dapivirine concentrations in plasma as an objective adherence marker. Here we describe and assess the effectiveness of this adherence monitoring system.

2.3 METHODS

Population and study procedures

The MTN-020/ASPIRE study was a randomized, double-blind, placebo-controlled phase III safety and effectiveness trial of a vaginal matrix ring containing dapivirine for the prevention of HIV-1 infection in African women (ClinicalTrails.gov number NCT01617096). Study design, methods and results are described elsewhere.[40,42] In brief, study enrollment began in August 2012 and was completed in June 2014 at 15 sites in four countries (Malawi, Uganda, South Africa, and

Zimbabwe); follow-up was completed in June 2015. At enrollment, participants were randomized in a 1:1 fashion to blinded active dapivirine vaginal ring or placebo. Thereafter, they attended monthly follow-up visits at which HIV-1 serologic testing, product resupply, and questionnaire-based assessments of sexual behavior and adherence were completed. Plasma samples to be used for measurement of dapivirine concentrations were collected and archived on a quarterly basis.

At the enrollment visit, participants were taught how to insert and remove the vaginal ring, and clinic staff confirmed via a digital examination that the ring was properly in place prior to the participant leaving the clinic. Clinic staff counseled participants to leave the ring in place throughout the four-week period between scheduled monthly follow-up visits and to return to the clinic with the ring in place at their next monthly visit. Participants were counseled to reinsert a clean ring any time it fell out or was taken out and to contact the clinic to receive a new ring if needed. Other than male or female condoms and tampons during menses, the participant was told to avoid use of all other vaginal products, since they could impact drug levels. Regular, ongoing ring use was stressed, and additional rings were provided if a participant anticipated not being able to attend a regularly scheduled monthly visit. All sites received institutional review board approval and participants provided written informed consent.

Rationale to initiate ongoing monitoring of adherence

Prior to initiation of MTN-020/ASPIRE, the study's leadership team determined that an adherence monitoring program concurrent to study implementation could prove beneficial to the overall conduct of the trial. Such a program would have to preserve blinding to arm assignments for investigators and participants, and otherwise maintain the overall integrity of the study data. Prior to implementation, a standardized approach to monitor adherence during the study by testing

plasma samples for dapivirine was designed and presented to the trial's independent Data and Safety Monitoring Board. During the first three months after the study initiated, the adherence monitoring system was commenced. Importantly, seven months after initiation of the MTN-020/ASPIRE study (March 2013), the results of the MTN-003/VOICE trial were reported publicly, revealing very low adherence to tenofovir-based PrEP as measured by objective levels of drug in blood plasma and reinforcing the need for an adherence monitoring strategy for MTN-020/ASPIRE.[5] Figure 2.7.1 displays a flow diagram of the steps involved and entities responsible for implementation of the real-time adherence monitoring system.

Specimen collection and shipping

The trial's data center staff created lists of aliquots of blood plasma specimens, referred to as specimen shipping lists. The shipping lists included both the active dapivirine and placebo arm participants and were based on specimens known in the trial's database to have been collected and archived from quarterly participant visits. The data center staff posted the shipping lists to a secure website on a monthly basis where core laboratory staff retrieved and sent them to the study sites. Site staff subsequently prepared shipments of plasma samples and sent them to the core laboratory.

Specimen selection and testing

Prior to January 2014, all available plasma aliquots from both the active and placebo arms were shipped from the core laboratory and tested at the pharmacology laboratory on a monthly basis. Due to cost considerations, in February 2014, the data center created testing lists containing randomly selected specimens by participant ID, visit and specimen ID, with approximately 90-95% of available specimens from active dapivirine arm participants and the remaining 5-10% from randomly selected specimens from placebo arm participants. The number of specimens was capped

at 500 initially and later at 750, the maximum affordable for testing in a month at the pharmacology laboratory. Core laboratory staff sent selected specimens to the pharmacology laboratory where dapivirine testing was completed. The relative proportions of active and placebo arm specimens were known only to the data center staff.

Laboratory testing methods

Plasma specimens were tested using a validated ultra-high-pressure liquid chromatographic-tandem mass spectrometric (UHPLC-MS/MS) method.[30] Lower (LLOQ) and upper (ULOQ) limits of quantification for the assay were 20 pg/mL and 10,000 pg/mL, respectively. Values below the LLOQ were reported as below the limit of quantification of the assay; no results were above the assay ULOQ. The assay was validated in accordance with the FDA, Guidance for Industry Bioanalytical Method Validation recommendations.[43]

Testing results processing

Pharmacology laboratory staff uploaded plasma test results to a secure data management system where data center staff downloaded, conducted data quality checks, and linked results to randomization arm assignments. Other members of the study team, including leadership and all site staff, did not have access to the raw or linked data. Instead, the data center staff summarized results showing only the percentages and no sample sizes from plasma samples among participants in the active arm where dapivirine was >20 pg/mL and >95 pg/mL for each study site, and overall. The >95 pg/mL level was chosen as an indicator for use of the vaginal ring for at least eight hours, and to exclude those who inserted the ring just prior to the study visit. Prior studies showed that plasma dapivirine levels reached >95 pg/mL with use of 8 hours or more.[37]

Testing results summarization

On a monthly basis, data center staff prepared summary reports of the plasma dapivirine results and communicated them to study leadership who, in turn, distributed the results to the study site leadership. The data were presented across calendar quarters overall, and by study site since interest was in changes in adherence behavior over calendar time. The first results were shared with study sites in March 2013 and continued on a monthly basis for the duration of the study. Figure 2.7.2 shows an example of how the tabular results were summarized. Study site leadership were given only their site's letter code and could see the relative ranking of their performance over time in a blinded fashion. Only this summary information was provided – no information on adherence by individual participants was given, either to study site teams or to the study leadership. At the study sites, adherence data were used to modify counseling messages, motivate staff, and create opportunities for participant engagement in the research process. As reported previously,[40] adherence data were also used to close enrollment at two sites where adherence and quality of study conduct was suboptimal.

Preserving the study blind

At each step, preserving study blind was considered essential and only the unblinded statistical staff within the data center had knowledge of the adherence results and randomization arm assignment of individual subjects. Initially when all samples were shipped, approximately equal numbers of specimens from active and placebo arm participants were tested making identification of arm assignment impossible. Later in the study, specimens in the placebo arm were randomly distributed throughout specimen testing lists using the parameters discussed previously, again making it impossible to identify a participant's arm assignment. The pharmacology laboratory staff who conducted the drug level testing had sample identifying information based on test results only

but did not have contact with the study sites and only shared results directly with the data center. Thus, study participants, clinical staff, site leadership, and study leadership remained blinded throughout the entire adherence monitoring process.

Communications within the study team

Regular, on-going communications between and within organizations was required. A weekly conference call was conducted between the core laboratory and the data center to work through any challenges in sample identification, testing, and data processing. Communications occurred regularly between the core, site and pharmacology laboratories, and data quality checks were on-going between the data center and laboratories. In addition, the protocol coordination center organized a monthly call between study and site leadership to discuss results.

Statistical analysis

To assess the feasibility of this adherence monitoring system, percentages of samples with dapivirine detected are summarized overall by quarterly calendar time. Plasma results from participants who were on a product hold or off product due to pregnancy are included. Changes in adherence to ring use are assessed over calendar time using generalized estimating equations with a logit link and exchangeable working correlation structure from all available plasma dapivirine test results. Analyses were conducted in SAS, version 9.4 (SAS Institute).

2.4 RESULTS

A total of 2,629 women were enrolled in MTN-020/ASPIRE. Their median age was 26 years (IQR 22-31), and median follow-up was 1.6 years (IQR 1.1-2.3). From October 2012 through January 2014 all quarterly plasma samples were shipped and tested; 4,618 total with 2,322 in the active

arm and 2,296 in the placebo arm. From February 2014 until the end of the study in June 2015 only selected samples were tested; 8,548 total with 7,036 in active arm and 1,512 in the placebo arm.

Adherence as defined by dapivirine levels of >95 pg/mL of plasma steadily increased across the 15 study sites (Figure 2.7.3). An upward linear trend was observed over calendar time, from 63% during Quarter 1 2013 to 84% during Quarter 1 2015 ($p < 0.0001$).

2.5 DISCUSSION

In this phase III, blinded, placebo-controlled study evaluating a vaginal ring containing 25 mg of dapivirine, a novel real-time adherence monitoring system was shown to be feasible and the feedback and corrective actions taken as a result of this feedback were temporally associated with improved adherence to vaginal ring use. Maintaining blinding to study product assignments for study participants, site staff, and study leadership was considered an essential aspect of the feasibility of this novel system and was shown to be possible. The success of the system also involved regular, on-going communication between the trial's data center, laboratories, study site staff, and study site leadership. To our knowledge, this is the first example to report on the process for and outcomes of real-time adherence monitoring in a blinded clinical trial of an HIV-1 prevention agent.

Other HIV-1 prophylaxis studies in African women have observed a strong relationship between adherence and HIV-1 protection, with two prominent studies finding no protection and very low adherence, which were only discovered after the trials were completed.[5,6] Those studies did not

conduct real-time adherence monitoring, thus did not have the opportunity to modify their approaches to actively promote adherence, as was the case in this study. The VOICE study, found that clinic-based product counts, face-to-face interviews and audio computer-assisted self-interviewing widely overestimated adherence (86%, 90%, 88% respectively) compared to post-hoc testing of tenofovir levels in plasma (<30%).[5] Qualitative examination in the VOICE study and other studies found several factors that influenced adherence to PrEP such as uncertainty of whether the investigational drug was efficacious, ambivalence about randomization that included a placebo, differences in participants' perceived HIV risk, uncertainty about use of an antiretroviral drug for HIV prevention, concerns about side effects, stigma, and partner support and/or acceptance of their participation in the study.[44-47] Moreover, motivation to take part in a phase III trial may have been based on interest in access to health services, and fees for participation rather than actual use of the investigational products, although participants were reluctant to disclose non-use due to fears of termination of current or disqualification from future research.[35] Additional qualitative studies showed that participants indicated that individual, social and structural factors were barriers to high adherence to PrEP,[46] and the use of a pictorial tool that supported discussion about drug levels between clinic staff and participants helped elucidate patterns of drug use and adherence behaviors.[35] Several of these qualitative studies concluded that the use of objective measures for adherence would result in a more accurate account of participants' true levels of use, and that evaluating objective measures of adherence in real-time were warranted.[5,34-36]

A qualitative sub-study conducted at 6 of the 15 study sites evaluated the acceptability of and adherence to the dapivirine vaginal ring.[48] Salient findings showed first that although participants initially feared use of the vaginal ring, they grew to like it and had a sense of ownership and ease of use over time. Second, support by staff and their peers aided in the uptake and sustained use of

the ring. Participants developed a sense of contributing to a common good and felt part of a team at their study site, due to blinded adherence feedback. Over time, the women developed a sense of ownership to a shared cause of contributing to a large-scale study of a novel HIV prevention tool with the potential to show efficacy and eventual licensure for future use in their communities. Last, male partner relationship dynamics were the most prevalent determinant for participants' acceptability and willingness to use the ring. If their partners were accepting of their participation, and participated in site activities promoting adherence, they were more likely to stay committed and adhere. These qualitative results support the notion that changes in adherence over study follow-up stemmed from the feedback provided by the real-time adherence monitoring system implemented in MTN020/ASPIRE.

An important goal in randomized, placebo-controlled phase III trials is to obtain an unbiased estimate of the true effect of treatment. Intention-to-treat analyses, the required primary analysis for regulatory submission of an investigational product, is based on participants' original randomization assignment, or equivalently, the policy of receiving one treatment versus another, regardless of adherence to assigned product, time off product, cross-over and loss to follow-up.[49] Adherence to assigned product during a phase III trial is, thus, a very important aspect of study conduct that ensures an intention-to-treat effect estimate is closest to a causal estimate. For example, when adherence to daily use of oral PrEP tenofovir-based products was shown to be high in four out of six trials in serodiscordant couples, injection drug users in Thailand, men who have sex with men, and heterosexual men and women intention-to-treat analyses were found to be statistically significant and ranged from 44-75%.[2-4,20] Adherence in these studies was >50% and a linear relationship between effectiveness estimates and adherence was seen. Furthermore, additional analyses among active arm participants where tenofovir was present in plasma showed

increased effectiveness estimates ranging from 85-100%.[2,4,33] Two trials, VOICE and FEM-PrEP, with detection levels of tenofovir in plasma of <30% were not able to show an effect in intention-to-treat analyses of similar products.[5,6] These results underscore the importance of improving adherence in phase III trials in order to provide an unbiased estimate of effect.

Notably, a statistically significant estimate of HIV-1 protection was found in MTN-020/ASPIRE. The overall effectiveness of the dapivirine vaginal ring in providing protection against HIV-1 infection over the duration of the trial was 27%, 37% after excluding the data from two sites with the poorest adherence to product and study visits, and 56% in women over 21 years of age.[40] In other HIV-1 studies of treatment and prevention it has been observed that use of product declines over time.[12,50] However, in this study, we observed an increase in product use in calendar time from 63-84% based on plasma measures, suggesting that real-time adherence monitoring could promote and sustain participant behaviors that lead to product adherence. The difference between this degree of adherence (63-84%) and the levels of HIV-1 protection seen (27-56%) could indicate that plasma dapivirine levels are an imperfect reflection of adherence, potential white coat dosing[51], or the maximum HIV-1 protection provided by the dapivirine vaginal ring, even with perfect use, may be less than 100%. Ongoing exploratory analyses are assessing the relationship between adherence and HIV-1 protection for this product.[40]

Whether it is feasible to monitor and counsel for adherence in the “real-world” roll out of PrEP is an important question to answer, and is currently under investigation in a phase IV, prospective study among young African HIV-uninfected woman.[52] Standard behavioral adherence support will be compared to an enhanced version including counseling based on real-time drug levels. The MTN-025/HOPE study, a phase IIIB open-label follow-on study examining safety and adherence

to the dapivirine vaginal ring is also underway.[53] Study staff are providing drug levels to participants choosing to use the ring starting one month from enrollment and until the 12-month follow-up visit. Furthermore, tenofovir urine testing was found to be effective for assessing adherence and work is currently underway to develop point of care urine assays.[54] These initiatives will help to answer the question of feasibility of monitoring and counseling for adherence in a "real-world" setting among women using PrEP.

There were limitations to this adherence monitoring approach. First, only the active arm participants' data was used in evaluating adherence. If a comparable placebo-arm adherence biomarker were available, estimates of overall ring use would have been possible. Second, although plasma dapivirine level is one objective measure of adherence, and the level of 95 pg/mL is a lower bound chosen based on previous phase 1 and 2 studies of the dapivirine ring,[31,37-38] this measure was likely not a perfect indicator of adherence across a full month, nor use around the time of HIV-1 exposure. Moreover, because women were expected to arrive for clinic visits with the ring in place, white coat dosing[51] could have signaled adherence for participants who were otherwise non-adherent. With further pharmacokinetic studies, improvements could be made to prevent misclassification, as has been done in clinical trials of tenofovir-based strategies.[50,55] Third, we measured plasma adherence levels only at quarterly visits, although ultimately our goal was to characterize participants' adherence profile throughout the monthly visit window. One measure does not suffice to adequately capture patterns of ring use over the entire quarter.

Women in Africa remain at high risk for HIV-1 and the dapivirine ring has been shown to provide protection in a phase III trial with concurrent implementation of an adherence monitoring system. Future large-scale effectiveness studies for HIV-1 prevention, including those for microbicidal

drugs delivered through a vaginal ring, should consider implementing a real-time adherence monitoring system to improve the validity of HIV-1 effectiveness estimates.

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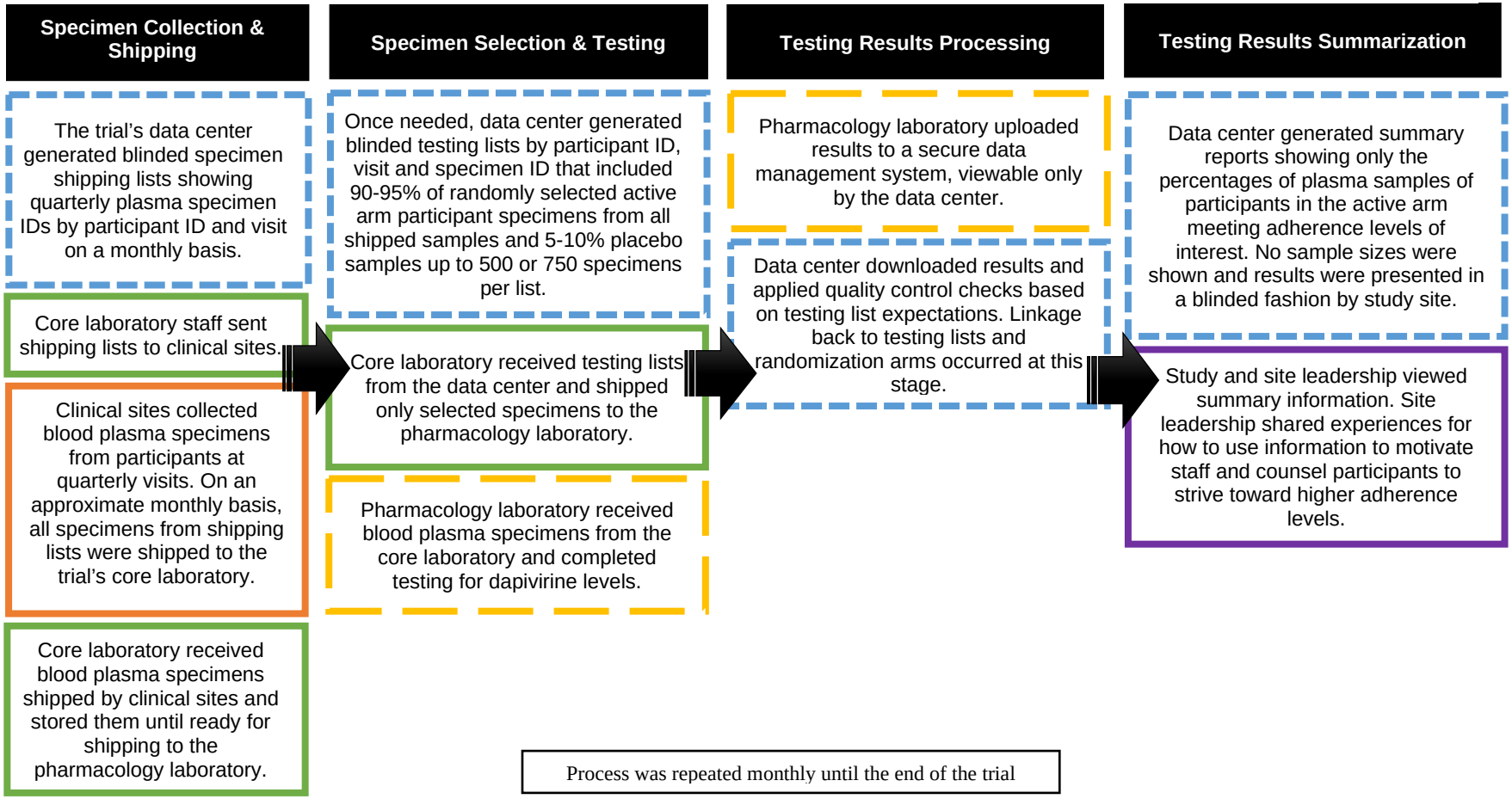
2.7 FIGURES

Figure 2.7.1 Flow Diagram of MTN-020/ASPIRE Real Time Adherence Monitoring System[1,2]



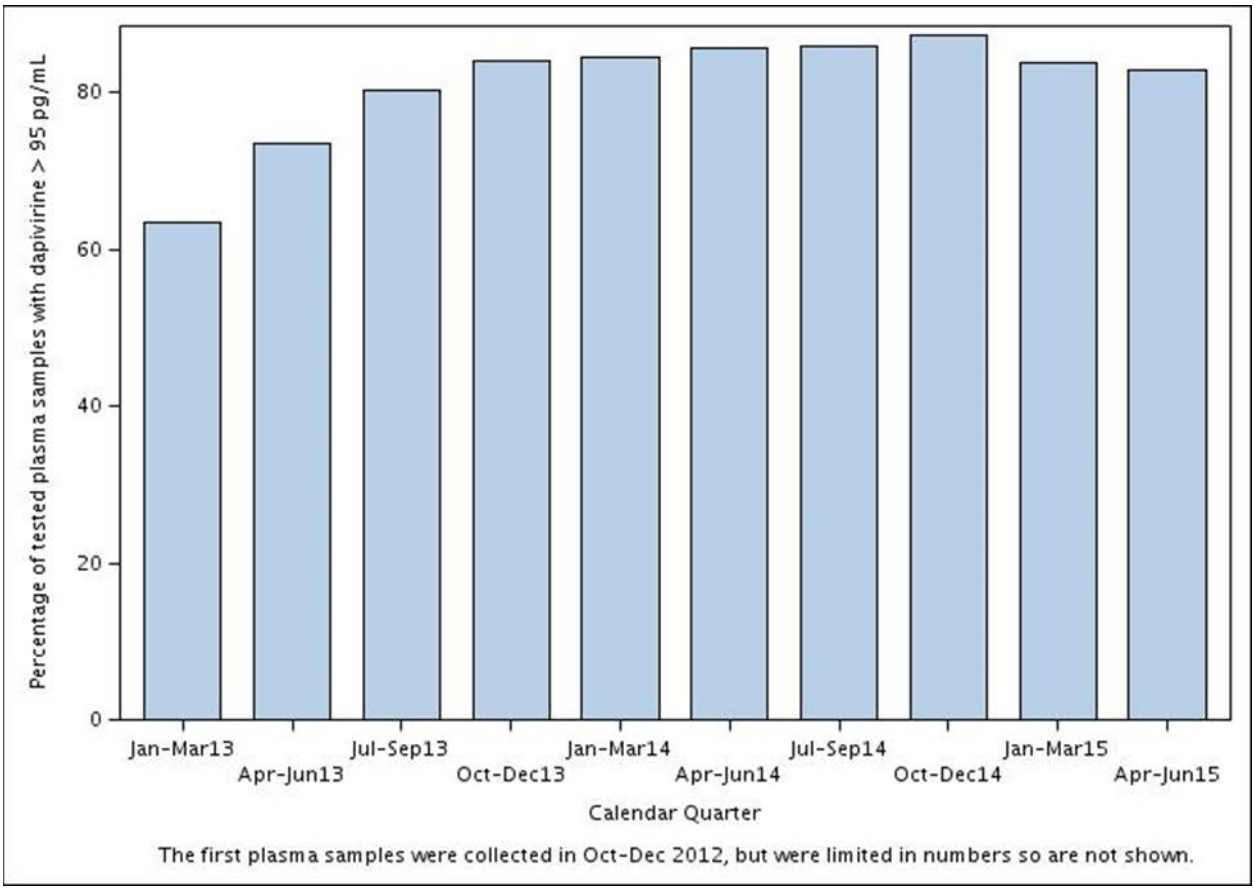
[1] Orange represents the clinical sites, blue represents the data center, green represents the core laboratory, yellow represents the pharmacology testing laboratory, and purple represents the MTN-020/ASPIRE study leadership team.

[2] Solid lines indicate that study blinding, defined as the identification of the linkage of participant identifiers (IDs) to the randomization arm, is upheld by staff for the organizations indicated by the color of the box. Small dashed lines indicate that randomization assignments are linked by participant ID so therefore staff are unblinded to the randomization assignment by participant ID. Large dashed lines indicate that the randomization arm may be deduced based on levels of blood plasma found through dapivirine test results so therefore staff were considered partially blinded to the randomization assignment.

Figure 2.7.2 Example of summary table presented to study sites – percentage of plasma samples among participants in the active arm with dapivirine >20 pg/mL and >95 pg/mL

Site	Jan-March 2013		…	April-June 2015	
	>20 pg/mL	>95 pg/mL		>20 pg/mL	>95 pg/mL
A	89	75		90	84
B	95	83		97	87
C	89	72		96	84
…	…	…	…	…	…
M	74	61		87	79
N	78	68		84	75
O	80	64		85	73
Total	82	63		89	84

Figure 2.7.3 Percentage of tested plasma samples among participants in the active arm across all 15 study sites with dapivirine >95 pg/mL by calendar quarter beginning January 2013



Chapter 3. EFFICACY OF THE DAPIVIRINE VAGINAL RING ACCOUNTING FOR IMPERFECT ADHERENCE: AN APPLICATION OF CAUSAL INFERENCE METHODS

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3.1 ABSTRACT

Adherence to product use is critical to obtaining unbiased estimates of efficacy of pre-exposure prophylactic interventions against HIV-1 infection. In the presence of imperfect adherence, intention-to-treat analyses assess a combined effect of adherers and non-adherers, and thus provide a biased and attenuated estimate of the causal effect of an intervention. Using data from MTN-020/ASPIRE phase III, placebo-controlled study evaluating HIV-1 efficacy of the dapivirine vaginal ring, we conducted per-protocol, and adherence-adjusted causal inference analyses using principal stratification and marginal structural models. We constructed two adherence cutoffs of ≥0.9 mg and >4.0 mg that represent drug released from the ring over a 28-day period. HIV-1 efficacy estimated from the per-protocol analysis was 30.8% (3.6%, 50.3%) (p = 0.03), and estimated from principal stratification analyses among the highest predicted adherers was 53.6% (16.5%, 74.3%) (p = 0.01) using 6-month data with the ≥0.9 mg cutoff. Marginal structural models produced efficacy estimates ranging from 48.8% (21.8, 66.4) *(*p = 0.0019) to 56.5% (32.8%, 71.9%) (p = 0.0002). Application of adherence-adjusted causal inference methods are useful in interpreting HIV-1 efficacy in secondary analyses of PrEP clinical trials.

KEYWORDS: Efficacy, adherence, HIV-1 prevention, dapivirine, intravaginal ring

3.2 INTRODUCTION

Adherence to product use is critical to obtaining unbiased estimates of efficacy of pre-exposure prophylactic (PrEP) interventions against HIV-1 infection. In clinical trials, an increasing number of PrEP strategies have been shown to be effective at reducing rates of HIV-1 infection, including evidence of very high HIV-1 protection when used with high adherence.[2-4] However, several clinical trials[5-7] that studied the use of oral tablets and topical vaginal forms of PrEP among women in sub-Saharan Africa failed to show high efficacy due to low adherence. For example, in the MTN-020/ASPIRE trial of the PrEP agent the dapivirine vaginal ring, intention-to-treat analyses showed a 27% reduction in HIV-1 risk but 37% after removing two less adherent clinical sites and 56% among women >21 years of age, who showed evidence of better adherence compared to their younger counterparts.[41]

Intention-to-treat analyses are the gold standard for estimating the average causal effect in interventional studies. However, in the presence of imperfect adherence to trial visits and/or products, intention-to-treat analyses are limited to assessing a combined effect of adherers and non-adherers, and thus provide a biased and attenuated estimate of the causal effect of an intervention. Many trials incorporate per-protocol analyses into their analysis plans, which estimate the average causal effect only over the time when treatment was actually received; however, such analyses could also be biased due to informative censoring.

Additional analytical approaches have aimed to provide unbiased estimates of efficacy in the context of incomplete follow-up and/or non-compliance to product use. These include the causal inference methods of principal stratification[21] and marginal structural models utilizing inverse-

probability-of-censoring weights and/or inverse-probability-of-treatment weights (IPCW and IPTW, respectively).[56] Principal stratification estimates efficacy among varying levels of adherers and allows for estimates of the complier average causal effect. Marginal structural models estimate efficacy among high adherers adjusting for confounding and selection bias. These methods could be applied to PrEP clinical trials data where non-adherence and informative loss to follow-up are present. We apply them to the MTN-020/ASPIRE study data with the aim of providing further understanding of HIV-1 efficacy from the dapivirine vaginal ring with high adherence, and to provide further understanding of the limitations of these methods to fully account for nonadherence.

3.3 METHODS

The MTN-020/ASPIRE study was a randomized, double-blinded, placebo-controlled, phase III trial among African women designed to test the safety and effectiveness of monthly use of a matrix intravaginal ring containing dapivirine, a non-nucleoside reverse transcriptase inhibitor (NNRTI). The trial was carried out at 15 clinical sites in Malawi, South Africa, Uganda, and Zimbabwe from August 2012 through June 2015. Details of the design, methods and results of the trial (ClinicalTrails.gov number NCT01617096) are reported elsewhere.[40,42] Institutional review boards at each participating clinical site approved the study protocol, and participants provided written informed consent for participation and specimen storage.

Population and study procedures

Sexually active, non-pregnant, HIV-1 seronegative women between the ages of 18-45 years were randomized in a 1:1 fashion to use of the vaginal ring containing 25 mg of dapivirine or a matching

placebo ring. At monthly visits, women were instructed to insert and wear the ring continuously until their next monthly visit. Clinic staff collected baseline demographic, and monthly sexual risk behavior, male partner characteristics, menstrual bleeding, vaginal practices, and adherence data on case reports forms. Testing for sexually transmitted infections including chlamydia, gonorrhea, trichomoniasis, and syphilis was done at baseline and post-randomization. Blood specimens were collected for HIV-1 serologic testing monthly and incident HIV-1 infections were adjudicated by an endpoints committee blinded to treatment assignment and to adherence.

Covariates used in models

Baseline covariates assessed as predictors of adherence in logistic regression models for principal stratification, and inverse-probability of censoring/treatment weighted marginal structural models were as follows: study site, age (18-21, 22-26, 27-45 years old), marital status (not married, married), highest level of education (no secondary education, some secondary education or higher), alcoholic drinks per week (none, 1-6, 7 or more), owns mobile phone (does not own a phone, owns a phone), travel time to clinic (<30 minutes, 30-60 minutes, 1-2 hours, >2 hours), earns own income (does not earn own income, earns own income), body mass index (underweight: ≤18.5, normal weight: 18.5-24.9, overweight: 25-29.9, obesity: ≥30), number of vaginal sex acts in past 3 months, received money/material goods/gifts/drugs/shelter in exchange for vaginal or anal sex in the past year (no, yes), anal sex (no, yes), any sexually transmitted Infections (chlamydia, gonorrhea, trichomoniasis, or syphilis; all negative, any positive), worried about having a vaginal ring inside of her every day for at least a year (not at all worried, somewhat worried, very worried). Covariates during follow-up included the following: percentage of vaginal sex acts protected by a condom in past 7 days [0%, 1-49%, 50-99%, 100% (or no sex)], male or

female condom use during last act of vaginal sex in past 7 days, anal sex in past 3 months (no, yes), number of male sex partners in past 3 months (0-1, 2, 3 or more), sex with a primary partner in past 3 months (no, yes, no primary partner), primary partner knows participant is taking part in the study (no/not sure, yes, no primary partner), primary partner knows participant is using vaginal ring (no/unsure, yes, no primary partner), same primary partner in past 3 months (no, yes, no primary partner), HIV status of primary partner in past 3 months (negative, positive, don't know, no primary partner), primary partner is taking antiretroviral drugs (no, yes, don't know, no primary partner), primary partner is circumcised (no, yes, don't know, no primary partner), bothered wearing ring every day (no, yes), number of times started or had her menstrual period in the last 3 months (0, 1, 2, 3), used something to control spotting or bleeding in last 3 months (no, no spotting/bleeding, yes), put anything inside vagina in last 3 months (no, yes), primary family planning methods [implants, intrauterine device, norethisterone enanthate, depot medroxyprogesterone acetate, other (includes oral contraceptive pills, sterilization, male or female condoms only, no family planning method)].

Measures of adherence

In a blinded fashion throughout follow-up, vaginal rings were collected and shipped to a central laboratory and tested for residual drug. Acetone extraction and high-pressure liquid chromatography was used to determine residual drug levels in the rings.[57] Vaginal rings from participants randomized to the dapivirine vaginal ring group only were used to construct two adherence cutoffs of ≥0.9 mg and >4.0 mg representing the amount of drug released from the ring over the expected 28-day monthly visit period. Manufacturing load levels minus residual drug levels were used to calculate the amount of drug released from each ring. Since study visits may

not have been spaced exactly to 28 days, the ratio of the amount of drug released to the number of days since dispensation was calculated and normalized to estimate the amount of dapivirine released during a standard 28-day period. A cut point of <0.9 mg was chosen to represent no or very low use of the ring, equating to one standard deviation of lab measurement error above zero mg dapivirine released based on testing of unused rings. We consequently defined an adherence outcome of moderate to high use of the ring at a level of ≥0.9 mg drug released during a 28-day period. In two phase I trials and one phase I/II trial,[37-38,58] investigators concluded that an average of 4.0-5.0 mg of dapivirine was released over a 28-day period of continuous ring use, and thus in this study >4.0 mg was also chosen as a more stringent measure, approximating continuous use.[59]

Estimating the average causal effect

The goal of this analysis is to present application of three statistical approaches (per-protocol, principal stratification, and marginal structural models) to a robust clinical trial dataset utilizing measures of adherence to a PrEP strategy, the dapivirine vaginal ring, and obtain estimates for the average causal effect under high adherence. A per-protocol analysis provides the net-treatment effect of the active drug adjusted for randomization assignment. The estimand of this analysis will be a biased estimate of the average causal effect if participants in the two treatment arms differ with respect to their compliance behaviors and HIV-1 infection status (i.e., the groups are not exchangeable). Conversely, by utilizing adherence measures, a principal stratification analysis will provide an estimate of the complier average causal effect, and the estimand can be denoted in general terms as follows: $E[Y(1)|X=1] - E[Y(0)|X=1]$ where $X=1$ is the sub-group of compliant participants, $Y(1)$ is the potential outcome for a participant receiving the active drug, and $Y(0)$ is the potential outcome for a participant when receiving placebo. The marginal structural model

analysis will utilize two IPCWs and an IPTW to estimate the average causal effect if all participants were to be continuously compliant to product use throughout the study.

Per-protocol analysis

In per-protocol analyses, we constructed two Cox proportional hazards (PH) models, each stratified by site with treatment arm (either dapivirine vaginal ring or placebo vaginal ring) included as the predictor. One of the models included additional covariates of age (<25 vs. ≥25 years old), marital status (married vs. non-married), number of sex partners in the last 3 months (0-1 vs. 2+), and baseline sexually transmitted infection status (defined as at least one positive result for chlamydia, gonorrhea, trichomoniasis, or syphilis vs. all negative results) found previously to be statistically significant predictors of HIV-1 infection in subgroup analyses and so were used to control for confounding.[40] Time to HIV-1 seroconversion was defined to mimic participant follow-up time as if accrued under perfect protocol adherence, censoring at the last negative HIV-1 test before detection of pregnancy or the first occurrence of three or more non-compliant events in a 12 month period that included the following: a missed visit, participants reporting the ring being out for more than 12 hours, and participant failing to return product to the clinical site. The event time was based on the time from enrollment to the earliest time of the following: last negative HIV-1 test prior to when the participant was determined to be pregnant, last negative HIV-1 test prior to the first occurrence of three or more non-compliant events in a 12 month period as stated above, HIV-1 infection, or the end of study follow-up. The censoring indicator was then based on the participant HIV-1 status at this newly defined event time. This analysis tested the hypothesis that there is no treatment effect using maximum likelihood estimates

and the score test. Estimates of the percent efficacy and 95% confidence intervals (CIs) are reported based on 1-hazard ratio obtained from Cox proportional hazards models.

Principal stratification analysis

We used potential outcomes [i.e., the pair of outcomes based on the treatment assignments to either placebo or dapivirine ring] of the variable adherence to ring use, to define "principal strata" that are independent of the treatment assignment. The principal strata adjust for characteristics inherent in the post-randomization variable of compliance without introducing selection bias as might be the case under a per-protocol analysis. The treatment effect estimated using this approach is therefore not affected by the post-randomization variable and so the estimand within the principal strata is considered to be causal. This method assumes the exclusion restriction for identifiability (i.e., potential drug non-compliers do not derive any treatment effect).[21] To differentiate compliers from non-compliers, we used the two measures of ring adherence of ≥0.9 mg and >4.0 mg of drug released over 28 days. Using the 6-month dataset, and 12-month dataset for sensitivity analyses, we constructed a logistic regression model using participant baseline characteristics among active arm participants only since adherence as defined by the amount of dapivirine released from the ring can only be known in the active arm. To build these models, we followed Shtatland, Kleinman, and Cain for finding the best predictive model utilizing a three-step process.[60] We used the logistic regression models to obtain the predicted probabilities of adherence among all participants in the ASPIRE cohort. Finally, we constructed a Cox proportional hazards model with treatment arm, the predicted probability of adherence and an interaction term between treatment arm and the predicted probability of adherence.[61] We calculated hazard ratios and 95% CIs to obtain percent

efficacy estimates for three strata defined by predicted probabilities of adherence of 0.86-0.90, 0.91-0.95, and 0.96-1.00, levels considered in this context to be relevant as treatment compliers.

Marginal structural models

In marginal structural models, a pseudo-population is created whereby participants are assigned weights proportional to their probability of being censored (due to pregnancy or informative loss to follow-up) and being compliant to treatment. We were interested in first re-weighting the population based on censoring due to pregnancy and informative loss to follow-up (i.e., the participant was terminated for one of the following reasons: investigator decision, refused further participation, unable to contact participant, or relocated with no follow-up planned). Second, we wanted to account for time-varying confounding of exposure to treatment and thus constructed weights based on the adherence outcome of ≥0.9 mg of released drug. The goal was to eliminate selection bias due to informative censoring that occurs when participants are non-adherent for reasons associated with being in the study.[56,62] Models fit in a pseudo-population which is equivalent to fitting a weighted model in the study population and the parameters of the weighted regression models, equal to marginal structural model parameters,[63] were used to estimate the average causal effect of the exposure in the original population. Thus, we constructed two separate IPCWs and one IPTW that were theoretically proportional to a subject receiving her own exposure history. We created stabilized IPCWs and IPTW[56] using baseline covariates only in the numerator, and baseline and time-varying covariates in the denominator using pooled logistic regression. Weights were then multiplied together, and a final weighted pooled logistic regression model was constructed to estimate the effect of dapivirine use among the re-weighted population. Weights in the placebo arm were set to 1.0 and all analyses excluded participant-visits in the first year of the

trial owing to missing residual drug levels in vaginal rings preventing estimation of weight models during that time period. All models censor participants at the time of first pregnancy (N=176) and include 131 out of 168 primary endpoints in the MTN-020/ASPIRE study. Thirty-seven endpoints were removed from the analysis due to missing data in IPCW and IPTW models. This analysis utilized monthly measures of ring adherence, and thus to align behavioral data collected on a quarterly visit schedule with monthly endpoint data, the last-value-carried-backward convention was used where appropriate. Analyses were conducted in SAS, version 9.4 (SAS Institute, Cary, North Carolina).

3.4 RESULTS

Table 3.7.1 displays baseline characteristics by randomization arm of the ASPIRE cohort included in this analysis. There were 2,629 participants randomized into the study, however 15 participants dropped out prior to the first visit where HIV-1 testing occurred. Therefore, a total of 1,308 in the dapivirine arm and 1,306 in the placebo arm were included in analyses. Approximately half of the participants were 27 years old or older, over 80% completed secondary school or higher, approximately 40% were married, the majority did not drink alcoholic drinks, over 90% owned a cell phone, and roughly half earned their own income.

Table 3.7.2 shows the results from the per-protocol analyses. Model 1 in this table was stratified by site and included only the randomization arm as a predictor for HIV-1 seroconversion. The estimated percent efficacy was 28.8% (95% CI: 0.9%, 48.9%; p = 0.04), and with additional adjustment for confounding was 30.8% (95% CI: 3.6%, 50.3%; p = 0.03). These estimates were

very close to those obtained from the intention-to-treat analyses of 27% (95% CI: 1%, 46%; p = 0.05).[40]

Table 3.7.3 and Figure 3.8.1 show the results of the principal stratification analyses. HIV-1 efficacy results for the first set of models utilizing the 6-month dataset to obtain the predicted probabilities of adherence of 0.86-0.90, 0.91-0.95, and 0.96-1.00 using the ≥0.9 mg cutoff to define adherence were all statistically significant at the $p < 0.05$ level and ranged from 29.7% (95% CI: 3.3%, 48.4%) to 53.9% (95% CI: 16.5%, 74.8%). Empirical estimates ranged from 22.3% (95% CI: 15.5%, 30.4%) to 48.2 (95% CI: 42.2%, 54.8%). For the 12-month dataset using the same cutoff for compliance, HIV-1 efficacy estimates ranged from 30.8% (95% CI: 3.3%, 50.6%) to 40.7% (-17.6%, 70.4%). Empirical estimates ranged from 14.2% (95% CI: 12.0%, 17.0%) to 59.9% (95% CI: 43.4%, 82.2%). Using the 6-month dataset and the >4.0 mg cutoff for adherence we found one, albeit unstable estimate, for HIV-1 estimated efficacy in the 0.86-0.90 predicted adherence group of 39.6% (95% CI: -3517.8%, 99.0%). Due to data sparsity for the remaining adherence groups using the 6-month and 12-month datasets, the estimated predicted probabilities and hazard ratios were inestimable in those regions.

For analyses utilizing IPCW and IPTW to construct marginal structural models, results are shown in Table 3.7.4. Models 1 and 3 show estimated percent HIV-1 efficacy using non-truncated stabilized weights for adherence alone, and the composite of adherence, first pregnancy and informative loss to follow-up, respectively. Models 2 and 4 show estimates using stabilized weights truncated at the 1%tile and 99th%tile for adherence alone, and the composite weight, respectively. HIV-1 efficacy across these four marginal structural models ranged from 48.8%

(95% CI: 21.8%, 66.4%) (Model 2) to 56.5% (95% CI: 32.8%, 71.9%) (Model 3). All estimates were statistically significant at levels of $p < 0.002$ or lower.

3.5 DISCUSSION

In this analysis of data from the MTN-020/ASPIRE randomized, placebo-controlled trial of the dapivirine vaginal ring, we used multiple analytical methods that account for imperfect protocol and product adherence to estimate HIV-1 protection efficacy. Per-protocol analyses, which aimed to mimic perfect protocol adherence, although without explicit consideration of product use, yielded efficacy estimates of approximately 30%, very near the intention-to-treat finding of 27% for the trial.[40] Causal models, specifically principal stratification and marginal structural, which considered both protocol and product adherence at the highest level for the ≥0.9 mg cut-off, estimated HIV-1 protection to be between 41% and 57%.

All analysis approaches we evaluated generated HIV-1 efficacy estimates that were higher compared to intention-to-treat, which was expected, given that retention and product adherence were known to be imperfect in the trial. Per-protocol analyses corrected for adherence by censoring at the time of first missed visits producing efficacy estimates only a few percentage points higher than those found in the intention-to-treat analysis. Such analyses did not account for selection bias due to informative censoring and assumed participants would have been fully compliant with product use. Principal stratification analyses aimed to categorize participants into sub-groups defined by predicted probabilities of adherence based on baseline characteristics. Although, assignment into strata that clearly defined highly adherent groups was difficult in this dataset due to the lack of strong predictors to adherence; the two residual drug release cut points to define a

participant as adherent were either arguably not stringent enough (≥0.9 mg) or overly stringent as evidenced by scarcity of data points (>4.0 mg). A study with a point-exposure instead of one that is time-dependent and user-dependent (as far as it affects consistency or misclassification of exposure), would have more likely met assumptions needed for marginal structural modeling. Compared to the other models, however, the marginal structural models likely provided closer estimates of true efficacy given they reweighted the dataset into pseudo-populations to account for time-varying confounding and selection bias at the same time.

By creating strata of exchangeable participants[23,65] considered to be moderately-high adherers (0.86-0.90), and strata considered to be very-high adherers (0.91-0.95 and 0.96-1.00) when using the ≥0.9 mg cutoff for adherence, we found monotonic increasing HIV-1 efficacy estimates for each of the 6-month and 12-month datasets. Notably, the empirical estimates and their 95% CIs trended similarly and were also contained within the 95% CIs of the predicted estimates. This is likely due to the additional statistical uncertainty from inclusion of the adherence parameter in the estimated models. Likewise, estimates of ring efficacy in the lower adherent group (0.86-0.90) had comparable estimates to those seen in intention-to-treat and per-protocol analyses. However, in the most adherent strata (0.96-1.00), we observed HIV-1 efficacy estimates ranging from 41% to 54%, albeit scarcity in the endpoints for the more stringent adherence groups resulted in highly imprecise or inestimable estimates. In marginal structural models, however, precision of efficacy estimates was not an issue, due to the large number of endpoints included in each model [although 22% (37/168) of endpoints were excluded from the analysis due to the unavailability of residual drug data from rings in the first year of the MTN-020/ASPIRE study]. The estimates ranged from 49% to 57%, depending on whether stabilized IPTW/IPCW's were truncated, and whether weights

were used for adherence alone, or a combined weight adjusting for adherence, first pregnancy or informative loss to follow-up.

Principal stratification analyses included two definitions of adherence based on release of drug in vaginal rings. The definition of ≥0.9 mg drug released during a 28-day period was considered representative of recent ring use, whereas >4.0 mg drug release, continuous use.[59] Considering the principal stratification efficacy estimates and their 95% confidence intervals using the 6-month dataset, the ≥0.9 mg drug release definition of adherence yielded estimates whereas those using the >4.0 mg drug release definition were inestimable due to data scarcity. This is not surprising since a less stringent definition of adherence includes a greater proportion of participants categorized as adherent, although likely misclassifies participants as adherent when they are not. The effect of exposure misclassification is typically attenuation of efficacy estimates. However, the group whose predicted probability of adherence is 0.96-1.00 likely contains participants who have the highest probability of adhering for the lower adherence threshold. Therefore, an efficacy estimate of 53.6% (95% CI: 16.5%, 74.3%) may provide a reasonable estimate of the complier average causal effect, since we can be more confident in this stratum that the exclusion restriction for identifiability assumption is being met. This assumption is based on the notion that potential non-adherers do not derive a treatment effect;[21] in our case, participants whose predicted probability of being adherent is very high at a lower threshold for adherence, is unlikely to include consistently non-adherent individuals given a cut point of <0.9 mg represents no or very low use of the ring. Likewise, this estimate is similar to that seen in subgroup analyses of women >21 years of age with an efficacy estimate of 56%, and who had evidence of better adherence compared to their younger counterparts.[40] Estimates at 12 months serving as sensitivity analyses, showed

attenuated point estimates although confidence intervals overlapped with the measures at 6 months. Of note, in the highest predicted probability of adherence group (0.96-1.00) there were relatively fewer HIV-1 endpoints (1.0 per 1000 person-months at 12 months vs. 4.2 per 1000 person-months at 6 months) possibly due in part to a study-wide temporal effect that was seen where adherence increased and began to level off at 12 months into the trial,[66] and thus numbers of seroconversions began to decrease due to increased adherence behaviors in both arms. Also, removing the first year of ring data from this analysis may have had an effect on the prediction models for categorizing participants into predicted adherence groups.

Several assumptions must be met for valid marginal structural models: 1) consistency (a participant's counterfactual outcome under her observed exposure history is precisely her observed outcome), 2) exchangeability (given measured confounders, the potential outcomes are independent of observed exposures; no unmeasured confounding, or ignorability of the treatment assignment and measurement of the outcome), 3) positivity (i.e., a nonzero probability of receiving every level of exposure for every combination of values of exposure and confounders that occur among individuals in the population), and 4) no misspecifications of the models (based on the constructed weights).[56,67] If assumptions are met, then the exposure is independent from the measured confounders in the pseudo-population. The consistency assumption asserts that a participant's exposure history is well-defined such that the potential outcome of a participant's exposure history is exactly that of the observed outcome.[68] However, in the case of exposure to the dapivirine ring, this assumption is clearly violated. As shown in the principal stratification analyses, we observed that inherent to the estimation of the complier average causal effect is determination of an appropriate cut point for adherence. It is clear that misclassification bias is

present since defining a perfect cut point, based on drug release to define sustained exposure to the ring that warrants protection, is unknown. Therefore, we are left with using cut points of the amount of drug released that are considered reasonable to define recent and continuous use of the ring. However, a more preferable definition to meet the consistency assumption would be one where exposure to the ring was completely controlled by the investigator such that consistent and sustained exposure over a 28-day period was guaranteed. Without this, one cannot confidently assume that the consistency assumption has been met. Second, the assumption of exchangeability, or no unmeasured confounding, may have also been violated. Although we included all potential confounders collected during the study when constructing the IPCW and IPTW models we cannot be completely confident other participant or study characteristics did not also play a role in adherence, pregnancy or loss to follow-up, and the outcome of HIV-1 infection. For example, other studies based on the MTN-020/ASPIRE cohort have shown that adherence improved over time when participants adjusted to wearing the ring and their fears of negative side effects dissipated,[48] or that participants initially were concerned about the novelty of the ring but over time developed a sense of comfort.[58,69-70] Although a variable pertaining to worries about ring use was included in the models, we did not adjust specifically for changes in perception or fears of use over time, which, if also related to the outcome of HIV-1 infection, could be considered an unmeasured confounder, or at least one with residual confounding. The additional assumption of positivity is reasonable since the MTN-020/ASPIRE cohort was large enough that over the distribution of covariates in the weight models, there were participants that did and did not have the outcomes (adherence, pregnancy and informative loss to follow-up). Finally, as in all modeling exercises, it could be that there was misspecification of models for baseline and time-varying covariates on

adherence to ring use, pregnancy and informative loss to follow-up as well as the final MSM models for effect of adherence to ring use on HIV-1 infection, controlling for baseline covariates.

Despite several limitations, the application of three statistical methods that attempt to account for violations of protocol procedures or varying levels of adherence produced higher HIV-1 efficacy estimates than those found in the primary intention-to-treat analyses of the trial. Obtaining estimation of efficacy of PrEP strategies such as the use of a vaginal ring for and among high-adherers can be valuable for the planning and implementation of future HIV-1 prevention studies. The application of several models in this analysis highlights the pros and cons of utilizing statistical methods derived from the potential outcomes framework for assessing efficacy in clinical trials in which both protocol and product adherence are imperfect.

3.6 ACKNOWLEDGEMENTS

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3.7 TABLES

Table 3.7.1 Baseline characteristics of MTN-020/ASPIRE participants

Characteristic	Dapivirine Ring N=1308 N (%)	Placebo Ring N=1306 N (%)
Age		
18-21	269 (20.6)	242 (18.5)
22-26	396 (30.3)	445 (34.1)
>=27	643 (49.2)	619 (47.4)
Education		
< Secondary school	212 (16.2)	191 (14.6)
>= Secondary school or higher	1096 (83.8)	1115 (85.4)
Married	525 (40.1)	544 (41.7)
Alcohol Drinks per Week		
None	1137 (87.0)	1158 (88.7)
1-6	139 (10.6)	120 (9.2)
7 or more	31 (2.4)	28 (2.1)
Owns a mobile phone	1179 (90.1)	1187 (90.9)
Earns own income	604 (46.2)	577 (44.2)
In the past year, received money, material goods, gifts, drugs, or shelter in exchange for vaginal or anal sex	73 (5.6)	88 (6.8)
Worried about having ring inside her every day for at least a year		
Not at all worried	921 (70.4)	918 (70.3)
Somewhat worried	366 (28.0)	370 (28.3)
Very worried	21 (1.6)	18 (1.4)
Positive for chlamydia, gonorrhea, trichomoniasis, or syphilis	284 (21.7)	259 (19.8)
Condom-protected vaginal sex in the last 7 days (no sex = 100% protected)		
0%	348 (26.6)	398 (30.5)
1-49%	64 (4.9)	67 (5.1)
50-99%	104 (8.0)	84 (6.4)
100%	792 (60.6)	756 (57.9)
Number of sex partners		
0-1	1097 (83.9)	1082 (82.9)
2 partners	156 (11.9)	147 (11.3)
3+ partners	55 (4.2)	76 (5.8)
Primary partner knows ppt. is taking part in study		
no/not sure	330 (25.2)	309 (23.7)
yes	972 (74.3)	990 (75.9)
no primary partner	6 (0.5)	6 (0.5)

Table 3.7.2 Results of Cox proportional hazards models for per-protocol analyses

Cox Proportional Hazards Model	Estimated Percent HIV-1 Efficacy	Estimated Percent HIV-1 Efficacy 95% CI	P-value
Model 1[1]	28.8	0.9, 48.9	0.04
Model 2[2]	30.8	3.6, 50.3	0.03

[1] This model was stratified by site and included randomization arm.

[2] This model was stratified by site and included randomization arm, and additionally the following variables to adjust for potential confounding: [age group (<25, ≥25 years old), marital status (married, not married), number of male sex partners (≥2 sex partners in last 3 months, 0-1 sex partners in last 3 months), sexually transmitted infections (any positive baseline result, all negative baseline results for the following: chlamydia, gonorrhea, trichomoniasis, or syphilis)].

Table 3.7.3 Principal Stratification: Estimated and empirical HIV-1 efficacy for predicted probability of adherence groups

Visit Month[1]	Adherence Cutoff (≥0.9 mg & >4.0 mg)	Number of Participants, Person-months of Follow-up, Number of HIV-1 Endpoints	Predicted Probability of Adherence[2]	Empirical HIV-1 Percent Efficacy[3]	Empirical HIV-1 Percent Efficacy 95% CI	Estimated HIV-1 Percent Efficacy[4]	Estimated HIV-1 Percent Efficacy 95% CI	P-value
6	≥0.9 mg	446, 9277, 34	0.86-0.90	22.3	15.5, 30.4	29.7	3.3, 48.4	0.03
6	≥0.9 mg	1375, 28615, 63	0.91-0.95	48.2	42.2, 54.8	42.9	13.2, 61.6	0.008
6	≥0.9 mg	143, 2858, 12	0.96-1.00	30.0	20.7, 43.5	53.9	16.5, 74.8	0.01
12	≥0.9 mg	557, 12262, 47	0.86-0.90	14.2	12.0, 17.0	30.8	3.3, 50.6	0.03
12	≥0.9 mg	692, 14526, 32	0.91-0.95	48.1	36.9, 60.3	36.3	-4.4, 60.5	0.07
12	≥0.9 mg	279, 5840, 6	0.96-1.00	59.9	43.4, 82.2	40.7	-17.6, 70.4	0.14
6	>4.0 mg	7, 144, 0	0.86-0.90	Inestimable[5]	Inestimable[5]	Unstable estimate[6]	Unstable estimate[6]	--
6	>4.0 mg	--	0.91-0.95	--	--	Inestimable[7]	Inestimable[7]	--
6	>4.0 mg	--	0.96-1.00	--	--	Inestimable[7]	Inestimable[7]	--
12	>4.0 mg	--	0.86-0.90	--	--	Inestimable[7]	Inestimable[7]	--
12	>4.0 mg	--	0.91-0.95	--	--	Inestimable[7]	Inestimable[7]	--
12	>4.0 mg	--	0.96-1.00	--	--	Inestimable[7]	Inestimable[7]	--

[1] Data from this visit month was used to construct the logistic regression models needed to obtain predicted probabilities of adherence based on best model using the Akaike Information Criterion (AIC). The number of participants included was as follows: 6-month model N=702, 12-month model N=1113.
[2] Predicted probabilities were obtained through logistic regression models using the adherence cutoff as outcome and baseline covariates as predictors.
[3] Garwood F (1936). Fiducial Limits for the Poisson Distribution. Biometrika 46:441-453.
[4] Efficacy estimates based on Cox proportional hazards models including arm, adherence group and arm x adherence group interaction term.
[5] There were 0 endpoints in each arm, so therefore the empirical estimate was inestimable.
[6] The HIV-1 percent efficacy for this adherence group based on the Cox proportional hazards model was: 39.6 (95% CI: -3517.8, 99.0; p=0.81).
[7] No participants had predicted probabilities of adherence at these levels and thus HIV-1 percent efficacy (95% CIs) was inestimable.

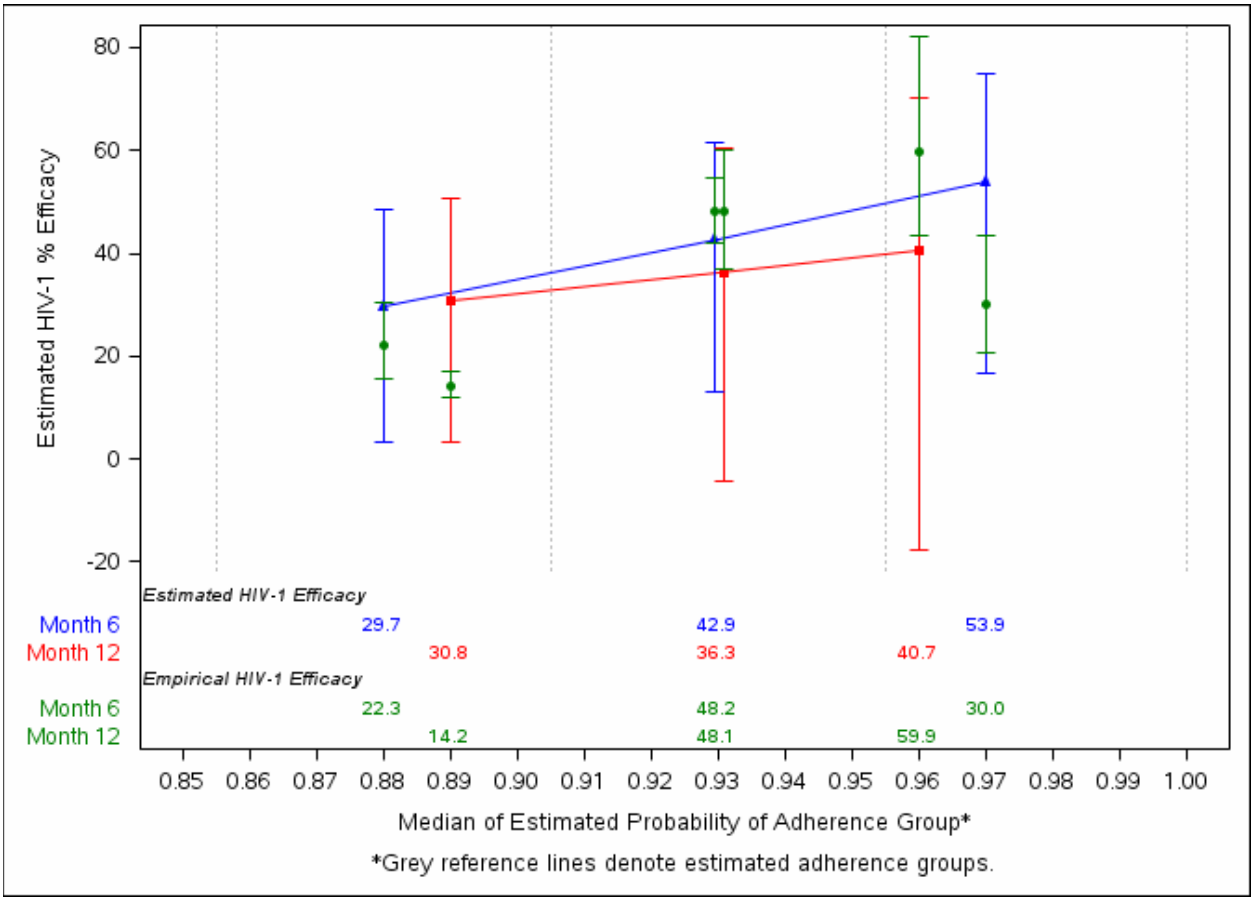
Table 3.7.4 Results of marginal structural models analysis

Generalized Estimating Equation Model	Number of HIV-1 Endpoints	Estimated Percent HIV-1 Efficacy	Estimated Percent HIV-1 Efficacy 95% CI	P-value
Model 1[1]	131	49.0	22.1, 66.6	0.0018
Model 2[2]	131	48.8	21.8, 66.4	0.0019
Model 3[3]	131	56.5	32.8, 71.9	0.0002
Model 4[4]	131	54.3	30.0,70.1	0.0003

[1] Using non-truncated stabilized weights for predicting adherence (in active arm only & using ≥0.9 mg cutoff), censored at time of first pregnancy.
[2] Using truncated stabilized weights (at 1%tile and 99th%tile) for predicting adherence (in active arm only & using ≥0.9 mg cutoff), censored at time of first pregnancy.
[3] Using non-truncated stabilized weights for predicting adherence (in active arm only & using ≥0.9 mg cutoff), time to first pregnancy and informative loss to follow-up. Data set was censored at time of first pregnancy.
[4] Using truncated stabilized weights (at 1%tile and 99th%tile) for predicting adherence (in active arm only & using ≥0.9 mg cutoff), time to first pregnancy and informative loss to follow-up. Data set was censored at time of first pregnancy.

3.8 FIGURES

Figure 3.8.1 Principal Stratification: Estimated and empirical HIV-1 efficacy for predicted probability of adherence groups (≥0.9 mg cutoff)



Chapter 4. CORRELATES OF ADHERENCE TO THE DAPIVIRINE VAGINAL RING FOR HIV-1 PREVENTION

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4.1 ABSTRACT

Background: Understanding the characteristics associated with adherence to pre-exposure prophylaxis (PrEP) methods for HIV-1 prevention may assist with optimizing implementation efforts for these products. The dapivirine vaginal ring is a novel topical PrEP delivery method.

Methods: Using data from a randomized, double-blind, placebo-controlled, phase III trial of the dapivirine vaginal ring conducted in four African countries, generalized estimating equation models were used to evaluate correlates of ring adherence. Two levels of quarterly dapivirine blood plasma (>95 pg/mL and >200 pg/mL), and dapivirine released from returned rings (≥0.9 mg and >4.0 mg) defined measures of adherence for recent and cumulative use, respectively.

Results: Time on study, calendar time, primary partner knowledge that the participant was taking part in the study, and use of long-acting contraceptive methods were associated with ring adherence whereas younger age, ring worries, condom use, episodes of menstrual bleeding and vaginal washing were associated with non-adherence.

Conclusions: These findings will be useful for recruitment into future clinical studies, real-time adherence monitoring and dapivirine ring implementation efforts. Evaluating multiple biomarkers of adherence provides a more thorough understanding of correlates of ring adherence and could strengthen the design of future adherence marker studies.

Keywords: Adherence, correlates, HIV-1 prevention, dapivirine, vaginal ring

4.2 BACKGROUND

In 2018, 37.9 million people worldwide were living with HIV-1, approximately half (18.8 million) of whom were women.[71] Since 2010, among those aged 15 or older, the annual number of new infections has remained essentially constant at an estimated 1.9 million.[72] In sub-Saharan Africa, young women accounted for 25% of new HIV-1 infections among adults and women of all ages accounted for 56% of new HIV-1 infections.[72] The use of pre-exposure prophylaxis (PrEP) for the prevention of HIV-1 infection has been shown to be highly effective at reducing rates of HIV-1 infection in women and men when used with good adherence.[2-4] However, in the absence of use, these products cannot protect against HIV-1.[5-7] Long-acting forms of PrEP could improve user adherence and HIV-1 protection.

In two phase III trials among women (Microbicides Trials Network (MTN)-020/ASPIRE and The Ring Study), a vaginal ring containing 25 mg of dapivirine, worn for 28 days, reduced HIV-1 risk by ~30% overall and in MTN-020/ASPIRE, by >50% among subgroups with greater adherence.[40,41] This study examines participant characteristics correlated with adherence to vaginal ring use in the MTN-020/ASPIRE study using objective adherence markers of dapivirine levels in plasma, and dapivirine released in rings.

4.3 METHODS

MTN-020/ASPIRE was a randomized, double-blind, placebo-controlled, phase III trial designed to test the safety and effectiveness of monthly use of a matrix vaginal ring containing dapivirine, a non-nucleoside reverse transcriptase inhibitor. The trial was carried out at 15 clinical sites in

Malawi, South Africa, Uganda, and Zimbabwe from August 2012 through June 2015. Details of the design, methods and results of the trial (ClinicalTrails.gov number NCT01617096) were previously reported.[40,42] Institutional review boards at each participating clinical site approved the study protocol, and participants provided written informed consent for participation and specimen storage.

Population and study procedures

Sexually active, non-pregnant, HIV-1 seronegative women aged 18-45 years were randomized 1:1 to use of a vaginal ring containing 25 mg of dapivirine or a matching placebo. At baseline, clinical staff collected demographic information and samples to test for sexually transmitted infections, including chlamydia, gonorrhea, trichomoniasis, and syphilis. In addition, on case report forms administered by study staff, participants were asked about the number of sex acts she had in the past three months, and whether she was worried about having the ring inside her every day for at least a year. At monthly visits, women were also asked about sexual practices with their male partners including condom use, partner knowledge of participation in the study and ring use, partner circumcision, partner HIV-1 status and antiretroviral use, menstrual bleeding patterns during ring use, vaginal practices such as douching, whether she was bothered wearing the ring daily, and adherence to the use of the vaginal ring. Responses about whether participants received money, material goods, gifts, drugs, or shelter in exchange for vaginal or anal sex in the past year were collected via an audio computer-assisted self-interview. Eligibility criteria included indication of use at enrollment (and intent to do so during study participation) of an effective form of contraception such as implants, intrauterine devices (IUDs), hormonal methods [i.e., intramuscular depot medroxyprogesterone acetate (DMPA-IM) and intramuscular injection

norethisterone enanthate (NET-EN)], and sterilization (defined as tubal ligation/hysterectomy/laparoscopy/other surgical procedure that causes sterilization). For the purpose of this analysis, family planning methods were categorized based on the primary form of contraception in use at the visit. If multiple family planning methods were reported, analysis deferred to the method that had been used the longest. The most prevalent methods such as implants, IUDs and hormonal methods were evaluated as potential correlates to adherence compared to all other reported methods such as oral contraceptive pills, sterilization, male or female condoms, or no method.

Participants were instructed to insert and wear the ring continuously until their next scheduled monthly visit. Rings were replaced at each monthly visit and blood specimens were collected for HIV-1 serology. At quarterly visits, blood plasma specimens were archived and subsequently tested for levels of dapivirine in active arm participants while preserving blinding of clinical staff and study participants.[66] Used rings were collected at monthly visits and sent to a central laboratory for residual drug testing. Residual drug measurements began approximately one year after the trial began due to a change from the initial plan of biofilm testing and the time needed for IRB approvals at the clinical sites. The processing involved for biofilms rendered rings from the first year of the study unusable for residual drug testing. Therefore, analyses using residual drug levels in rings include participant-visits after the first year of the study.

Measures of adherence

Among women randomized to the dapivirine vaginal ring arm only, two biological measures were used to construct measures of adherence at quarterly visits, as follows: dapivirine levels in blood

plasma (>95 pg/mL and >200 pg/mL), and the amount of dapivirine released from returned rings, based on residual drug levels remaining in the ring, over the expected 28-day monthly visit period (≥0.9 mg and >4.0 mg). For plasma, samples were sent to a central laboratory where testing employed ultra-high performance liquid chromatographic-tandem mass spectrometric methods to determine dapivirine levels.[30] A >95 pg/mL cut-point was observed in phase I/II trials to represent a level achieved with eight hours or more of continuous use; thus, >95 pg/ml represents a level that excludes those who inserted the ring just before the study visit. This level, however, could misclassify a woman as adherent who did not wear the ring consistently throughout the month but inserted the ring eight hours or more prior to the clinic visit (akin to "white-coat dosing").[51] Moreover, this level could also represent consistent use during the 28-day period and removal from 1-3 days prior to the clinic visit.[37-38] A more stringent plasma cut point of >200 pg/mL representing a higher bar for consistency of ring use compared to the >95 pg/mL level was also chosen. This level is based on evidence in a phase I/II trial that showed mean plasma levels of 293.4 pg/mL and 238.9 pg/mL over 4 and 12 weeks, respectively, among 140 participants in the dapivirine ring arm. Overall mean number of days of ring use was 83.3 (± 4) days.[58] Likewise, in two other studies of the same ring examining the pharmacokinetics of dapivirine, investigators found that with consistent use over a 24-hour period plasma levels of dapivirine were >200 pg/mL.[37-38]

Acetone extraction and high-pressure liquid chromatography were used to determine residual drug levels in the rings.[57] Manufacturer ring load levels of dapivirine minus residual drug levels were used to calculate the amount of drug released from each ring. Since study visits may not have been spaced exactly to 28 days, the ratio of the amount of drug released to the number of days since ring dispensation was calculated and normalized to estimate the amount of dapivirine released

during a standard 28-day period. A cut point of <0.9 mg was chosen to represent no or very low use of the ring, equating to one standard deviation of lab measurement error above 0 mg dapivirine released based on testing of unused rings. We consequently defined an outcome for moderate to high use of the ring at a level of ≥0.9 mg drug released during a 28-day period. In two phase I trials and one phase I/II trial,[37-38,58] investigators concluded that an average of 4.0-5.0 mg of dapivirine was released over a 28-day period of continuous ring use, and thus in this study >4.0 mg was also chosen as a more stringent measure, approximating consistent use.[59] Results from quarterly visits, instead of monthly visits, beginning one year after study start are used to be consistent with the timing of the blood plasma outcomes.

4.4 STATISTICAL ANALYSIS

Four separate generalized estimating equation (GEE) models with a logit link and exchangeable working correlation structure were built for participants randomized to the dapivirine ring arm only using a three-step process. Step one entailed constructing univariable GEE models for all potential correlates (data not shown), including baseline, demographic and time-dependent behavioral factors, to determine covariates to be included in building a multivariable model. Based on statistical significance of $p \leq 0.05$ in univariable models and findings from previous analyses[40,66] the following variables were selected to be included in all multivariable models: clinical site, age, time on study, and calendar time. In step two, a high-performance statistical modeling selection routine[73] was employed using variables determined in step one. After inclusion of the four variables described in step one, the routine assessed each additional covariate based on $p \leq 0.20$ for entry and $p \leq 0.10$ to remain in the model. Note that the standard error for the estimated beta coefficients using this model selection routine did not take into account the intra-class

correlation in measures. However, we expect the routine to provide more inclusive covariate selection since the standard errors of the beta estimates would be overestimated compared to when adjusting for intra-class correlation in the measures. We considered this approach acceptable given the large number of covariates tested and our objective of discovering any potential correlate to adherence for future hypothesis generation. Once candidate covariates were determined, step three involved constructing a final multivariable GEE model with a logit link function and exchangeable working correlation structure for each outcome using all variables identified in step two. Statistical analyses were completed on participant-visits whereby at least one vaginal ring was previously dispensed. Rings not returned to the clinical sites were considered as not used and if a participant refused the use of a ring, she was classified as non-adherent. Associations are shown as odds ratios (OR) with their corresponding 95% confidence interval (CI). P-values were not adjusted for multiple comparisons since this analysis was exploratory in nature. Analyses were conducted in SAS, version 9.4 (SAS Institute, Cary, North Carolina).

4.5 RESULTS

As shown in Table 4.8.1, 1,313 women were randomized to the dapivirine arm of MTN-020/ASPIRE. The present analysis includes data from 1,273 (97%) participants with 8,639 quarterly participant-visits for the model with plasma >95 pg/mL as the adherence outcome, and 8,610 quarterly participant-visits for the model with plasma >200 pg/mL as the adherence outcome. Models using the amount of drug released from rings as the adherence outcome included 1,218 (93%) participants, with 7,222 quarterly participant-visits using the ≥0.9 mg cut point and 7,035 quarterly participant-visits using the >4.0 mg cut point. Participant follow-up was 12 to 33 months. Among non-missing plasma results from quarterly participants-visits 82.8% had DPV

levels >95 pg/mL, and 49.2% had DPV levels >200 pg/mL. Among non-missing rings with quantifiable residual drug levels at quarterly visits 85.7% had DPV ring release levels of ≥0.9 mg, and 26.3% had DPV ring release levels of >4.0 mg.

Among women randomized to the dapivirine ring arm, at baseline, approximately half were 18-26 years old, most had some secondary education or higher, and more than half were not married. Nearly all reported a primary partner in the three months prior to enrollment; three-quarters of participants' partners were aware of their participation in the study. Approximately half (46%) reported at baseline that they used a condom and 30% reported no condom use during their last act of vaginal sex in the past seven days, with the remainder reporting no recent sex (24%). Approximately 30% of participants reported at baseline that they were somewhat or very worried about wearing the vaginal ring every day for a year. In the prior three months, 77% used something (such as tissue, toilet paper, cloth, cotton wool, tampon, sanitary pad, water with or without soap) to control menstrual-cycle related spotting or bleeding, and 60% put something inside their vagina (such as for vaginal washing or drying). Approximately 6% received money, material goods, gifts, drugs, or shelter in exchange for sex in the previous year. Twenty-two percent were found to have a sexually transmitted infection at baseline: chlamydia (13%), gonorrhea (4%), trichomoniasis (7%), or syphilis (1%). At baseline, DMPA-IM was the most commonly used family planning method (39%), followed by implants (19%), the intramuscular injection NET-EN (15%), intrauterine devices (12%) and other forms of contraception such as oral contraceptive pills (10%) or sterilization (3%).

In multivariable analyses, longer time in follow-up for participants and later calendar time on study were significantly positively associated with plasma adherence outcomes of >95 pg/mL and >200 pg/mL. However, this result was not replicated in the outcomes based on the amount of dapivirine released from the ring. Younger age was negatively associated with the plasma adherence outcomes of >95 pg/mL and >200 pg/mL, and the outcome of ≥0.9 mg DPV released from the ring. As shown in Table 4.8.1, compared to those women ages 27-45 years, 18-21 and 22-26 year olds were less likely to be adherent with ORs of 0.55 (95% CI: 0.40, 0.77) and 0.61 (95% CI: 0.47, 0.80), respectively, for the outcome of plasma >95 pg/mL, and 0.72 (95% CI: 0.58, 0.88) and 0.86 (95% CI: 0.72, 1.03) for the outcome of plasma >200 pg/mL. Likewise, the ORs were 0.66 (95% CI: 0.47, 0.92) and 0.65 (95% CI: 0.49, 0.86), respectively, for the outcome of ≥0.9 mg dapivirine released from the ring.

Additional baseline variables found in model selection for the plasma >95 pg/mL outcome were the number of alcoholic drinks per week, travel time to the clinic, whether the participant earns her own income, and body mass index (BMI). For the plasma >200 pg/mL outcome, other baseline covariates included in the model were education, the number of alcoholic drinks per week, and whether the participant owns a mobile phone. For the outcome of ≥0.9 mg dapivirine released from the ring, seven or more alcoholic drinks per week compared to no drinks per week was found to be marginally associated, whereas higher BMI compared to being underweight was marginally associated with the >4.0 mg dapivirine released from the ring outcome.

An increase by one in the number of vaginal sex acts in the prior three months from baseline was marginally negatively associated with the plasma >95 pg/mL adherence outcome. Time-dependent

condom use and no condom use during the last act of vaginal sex in the past seven days compared to no sex were marginally negatively associated with both plasma outcomes and the >4.0 mg dapivirine released from the ring outcome. Primary partner knowledge of participants involvement in the study over time was positively associated with both plasma adherence outcomes. Primary partner knowledge that the participant is using the vaginal ring was selected into the plasma adherence models only and was marginally negatively associated with the plasma >200 pg/mL outcome. Compared to not having the same primary partner in the past three months, participants reporting no primary partner or those having the same primary partner was associated with the outcome of >4.0 mg dapivirine released from the ring with ORs of 1.43 (95% CI: 0.95, 2.15), and 1.73 (95% CI: 0.99, 3.04), respectively. For the plasma >95 pg/mL outcome, the association was in the opposite direction with ORs of 0.64 (95% CI: 0.43, 0.95) for those reporting that they had the same primary partner and 0.75 (95% CI: 0.42, 1.35) for those reporting no primary partner in the past three months, respectively.

Whether the participant was somewhat worried about having the ring inside her every day for at least a year compared to not being worried at all was negatively associated with the plasma adherence outcomes [ORs: 0.72 (95% CI: 0.55, 0.92) for the >95 pg/mL outcome, and 0.87 (95% CI: 0.72, 1.04) for the >200 pg/mL outcome]. Reporting more menstrual bleeding episodes in the last three months compared to no bleeding was marginally associated with lower adherence for the plasma >200 pg/mL outcome [ORs: 0.85 (95% CI: 0.71, 1.01) for one episode, 0.91 (95% CI: 0.78, 1.07) for three episodes], and statistically significantly associated with lower adherence for the outcome of >4.0 mg dapivirine released from the ring [ORs: 0.79 (95% CI: 0.63, 0.98) for two episodes, and 0.75 (95% CI: 0.60, 0.95) for three episodes]. Participants reporting having put

something inside the vagina in the last three months compared to not were less likely to be adherent for all outcomes. Whether a participant reported receiving money, material goods, gifts, drugs, or shelter in exchange for vaginal or anal sex was marginally negatively associated with the >95 pg/mL plasma outcome [OR: 0.70 (95% CI: 0.47, 1.06)]. A positive test result for chlamydia at baseline showed a marginal negative association for the plasma >200 pg/mL outcome only [OR: 0.85 (95% CI: 0.69, 1.05)].

The use of implants, NET-EN, or DMPA-IM as the primary family planning method compared to a combined reference group of oral contraceptive pills, sterilization, male or female condoms, and no family planning method were each statistically significantly positively associated with adherence for all outcomes. The use of an IUD compared to the reference group, however, showed marginal positive association with the ≥0.9 mg dapivirine released from the ring outcome, and a marginal negative association with the >200 pg/mL plasma outcome, and the >4.0 mg dapivirine released from the ring outcome.

4.6 DISCUSSION

In this study of a 25 mg dapivirine vaginal ring, a novel topical HIV-1 PrEP agent, demographic, behavioral, and time-dependent factors were related to objective measures of adherence. Our analyses suggest that ring use improved over time for individual women as measured by plasma outcomes, possibly indicating improving acceptance, better understanding of proper use, and improving partner acceptability.

The significant independent positive association between adherence and the use of longer-acting hormonal-based family planning methods was a result not directly seen in other studies of the dapivirine ring. Although, in one study comparing stated preference between tablets, injections and the ring, ring use was preferred among women reporting use of implants or IUDs.[74] Women reporting use of longer-acting methods were more likely to adhere to ring use, which was broadly seen for all adherence markers. This could be due to participants being early adopters of new technology and thus more willing to adopt the use of a novel strategy like the ring.[74] Or, perhaps women who had experience using an implant for a period of time were more comfortable with the use of another internal device such as the ring as compared to women who did not have experience with that type of longer-acting contraceptive method.[75] It is possible, and further research is warranted, to examine whether women who were willing to use longer-acting contraceptive methods made more prevention-oriented decisions, and thus, may have been less concerned about an unintended pregnancy occurring while using the investigational ring. Conversely, women who used an intrauterine device may have had concerns about integrating another device internally. One study evaluated the effect of dapivirine exposure on the effectiveness of hormonal contraception, but did not evaluate its influence on adherence to the use of the ring.[76] Although, notably, a qualitative analysis of a subset of MTN-020/ASPIRE participants found that perception of "vaginal overload" with tampon, intrauterine contraceptive device, and ring use during menses was an impetus for ring removal.[77]

Our findings of the association of time on study, and calendar time, with adherent use of the vaginal ring as measured by plasma levels of dapivirine are consistent with prior exploratory analyses conducted in the MTN-020/ASPIRE cohort.[40] Qualitative work in MTN-020/ASPIRE found that

participants became more comfortable with wearing the ring once they adjusted to using it and fears of negative side effects dissipated with counseling and support from study staff and peers over time.[48] Other studies support the hypothesis that participants initially may have concerns about the novelty of the ring and over time developed a sense of comfort.[58,69-70] The association with calendar time likely reflects, in part, implementation of a real time adherence monitoring intervention, as previously reported.[66] Of note, used rings were not available for this analysis from the first year of the study, which may explain why a similar positive trend was not seen for the ring residual drug outcomes. Our findings are in stark contrast to other HIV-1 prevention and treatment strategies in trials that have found waning adherence over time and may suggest favorable persistence to the dapivirine vaginal ring, once initial barriers to use are overcome.[12,50]

Some participant characteristics appeared to have played a role in adherence to use of the ring. Younger age compared to older age is associated with less consistent adherence to ring use, as was seen in earlier analyses from MTN-020/ASPIRE and in other PrEP studies.[40-41,77] This finding is consistent with studies showing that younger women may have challenges maintaining full adherence to HIV-1 treatments, prevention agents, and other similar user-dependent strategies like contraceptives.[46,78-80] Importantly, our finding suggests that some younger persons will need additional adherence support in order to realize the ring's HIV-1 risk reduction benefits. Moreover, safety of ring use, perhaps due to intentions to get pregnant and fear of effects on a future pregnancy, should be further studied since unproven safety of an investigational product could be a possible explanation for the observed reduction of adherence among young participants in the MTN-020/ASPIRE cohort.

Other participant characteristics such as the higher number of alcoholic drinks per week and higher BMI were suggestive of lower adherence with respect to the drug release marker, in particular. The finding that partner knowledge of the participant taking part in the study is associated with higher adherence to the ring is consistent with qualitative findings indicating that when participants disclosed their participation in the study to their male partners they were often met with support to use the ring and appreciation for how the ring might help them or their families in the future.[48] In serial in-depth interviews, participants reported that their partners' attitudes changed over time from one of concern or opposition to greater acceptance and use of the ring. Qualitative results also showed that some women were proactive in removing the ring prior to sex, and preemptively disclosed ring use to their partners to avoid inciting abusive behavior that might follow inadvertent disclosure.[48] Acceptability measures related to interference with sex were also found to be associated with non-adherence to ring use.[81] Moreover, women who were in stable relationships disclosed ring use to obtain acceptance by their primary partners, whereas women who were not married, or their relationship was new, transactional or casual were less likely to disclose to partners that they were using the ring.[48] Our finding that adherence was lower with any condom use compared to no sex is suggestive that women may have been less interested in ring use if other HIV-1 protection methods were being used, or simply that they removed the ring prior to sex whether a condom was used or not.

We found that transactional sex, testing positive for chlamydia and ring worries at baseline, and number of menstrual bleeding episodes in last 3 months, partner circumcision and putting anything inside the vagina in the last three months also played a role in whether women were adherent to the use of the ring. It is perhaps not surprising that women who were worried about using the novel

ring were less likely to use the ring on a regular basis, as was seen in a sub-study examining ring worries among MTN-020/ASPIRE participants.[82] Women who experienced more menstrual bleeding episodes or who put things in their vagina during the month may have perceived that the use of the ring was interfering. Previous qualitative findings corroborate these results: some women reported that they removed the ring during menses because they felt unclean or wanted to wash it. Likewise, women reported that they would remove the ring if they were concerned with side effects such as vaginal discharge, odor, or itching to see if symptoms resolved.[48] In another MTN-020/ASPIRE qualitative sub-study, reasons for ring removal included hygiene, beliefs that the ring blocked the flow of menstrual blood, and fears that the ring would come out with blood or during tampon removal.[83]

Although this analysis proved useful for identifying possible correlates to adherence to ring use, there were several limitations. First, there is no perfect objective measure of dapivirine vaginal ring use and thus there is potential for misclassification of the outcomes.[57] However, we believe that objective markers like plasma and ring residual drug levels as measures of adherence are likely to provide a more sensitive estimate for women's true level of adherence than self-reported measures.[77] Therefore, our analysis likely provides reasonable hypothesis-generating results especially given they show congruence with qualitative findings on perceived factors associated with adherence (e.g., sexual behavior, menses, partner disclosure, ring worries, use of contraceptive methods, etc.). Second, given the quantity of covariates to be assessed, constructing all possible subsets of models was impossible and thus we relied on a high-performance modeling routine to generate the best multivariable models. Last, misclassification bias may be present for any one of the adherence outcomes impacting which covariates were ultimately selected in the

multivariable models. Measurement error and misclassification of the outcomes, assuming present at both levels and non-differential (exists at the same level for the outcomes for all levels of the covariates) would result in attenuation of odd ratios (i.e., toward the null). However, given that we examined four different outcomes, two objective measures with low and high bars to define adherence, we believe this approach may have also provided a more robust insight into correlates when for example we found similar effects across the adherence outcomes.

Correct and consistent use of the dapivirine vaginal ring, like other PrEP (and HIV-1 treatment) interventions, is key to effectiveness. Future studies examining similar vaginal rings could benefit from knowing characteristics of women that are correlates to adherence. This new knowledge could be used in recruitment into future clinical studies, real-time adherence monitoring and dapivirine ring implementation efforts. Evaluating multiple biomarkers of adherence provides a more thorough understanding of correlates of ring adherence and could strengthen the design of future adherence marker studies. Likewise, these results may help inform implementation of the dapivirine vaginal ring were it to be approved for use in high HIV-1 prevalence settings.

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MTN-020/ASPIRE Study Team

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4.8 TABLES

Table 4.8.1 Correlates of adherence to the dapivirine vaginal ring, summary of multivariable models[1]

		Plasma DPV >95 pg/mL	Plasma DPV >200 pg/mL	DPV Released from Ring ≥0.9 mg	DPV Released from Ring >4.0 mg
Characteristic[2]	**N (%) at Baseline (Total N=1,313)**	**OR (95% CI) (Participants: N=1,273, Participant-visits: 8,639)**	**OR (95% CI) (Participants: N=1,273, Participant-visits: 8,610)**	**OR (95% CI) (Participants: N=1,218, Participant-visits: 7,222)**	**OR (95% CI) (Participants: N=1,218, Participant-visits: 7,035)**
Time					
Time on Study					
3- & 6-Month Visit		Ref	Ref	Ref	Ref
9- & 12-Month Visit		1.33 (1.14, 1.54)	1.12 (1.00, 1.26)	1.03 (0.86, 1.23)	0.95 (0.80, 1.11)
15 through 33 Month Visit		1.33 (1.08, 1.64)	1.35 (1.17, 1.56)	0.90 (0.72, 1.13)	0.99 (0.82, 1.20)
Calendar Time					
Aug2012-Dec2012		Ref	Ref	N/A[3]	N/A
Jan2013-Dec2013		1.87 (1.10, 3.20)	2.07 (1.19, 3.60)	Ref	Ref
Jan2014-Dec2014		2.56 (1.47, 4.49)	2.30 (1.31, 4.04)	1.08 (0.90, 1.30)	1.20 (1.01, 1.42)
Jan2015-June2015		2.09 (1.17, 3.71)	3.29 (1.86, 5.84)	0.92 (0.72, 1.18)	0.91 (0.73, 1.13)
Baseline Characteristics					
Age					
18-21	269 (20.5)	0.55 (0.40, 0.77)	0.72 (0.58, 0.88)	0.66 (0.47, 0.92)	0.93 (0.74, 1.17)
22-26	396 (30.2)	0.61 (0.47, 0.80)	0.86 (0.72, 1.03)	0.65 (0.49, 0.86)	0.93 (0.77, 1.13)
27-45	648 (49.4)	Ref	Ref	Ref	Ref
Education					
No secondary education	212 (16.2)	N/A	Ref	N/A	N/A
Some secondary education or higher	1101 (83.9)		0.89 (0.70, 1.13)		
Marital Status					
Not Married	786 (59.9)	N/A	N/A	N/A	N/A
Married	527 (40.1)				
Alcoholic Drinks Per Week[4]					
No drinks	1141 (86.9)	Ref	Ref	Ref	Ref
1-6 drinks	139 (10.6)	1.16 (0.81, 1.65)	1.19 (0.93, 1.51)	0.90 (0.61, 1.34)	0.88 (0.66, 1.17)
7 or more drinks	32 (2.4)	0.68 (0.36, 1.28)	0.70 (0.42, 1.17)	0.52 (0.26, 1.04)	0.64 (0.37, 1.13)
Owns Mobile Phone					
Does not own a phone	129 (9.8)	N/A	Ref	N/A	N/A
Owns a phone	1184 (90.2)		1.16 (0.88, 1.52)		
Travel Time to Clinic					
<30 mins	306 (23.3)	0.77 (0.33, 1.81)	N/A	N/A	N/A
30-60 mins	655 (49.9)	0.90 (0.40, 2.05)			
1-2 hours	310 (23.6)	0.86 (0.38, 1.95)			
>2 hours	42 (3.2)	Ref			
Earns Own Income					
Does not earn own income	707 (53.9)	Ref	N/A	N/A	N/A

Earns own income	606 (46.2)	0.92 (0.71, 1.20)			
Body Mass Index[4]					
Underweight: ≤ 18.5	41 (3.1)	Ref	N/A	N/A	Ref
Normal weight: = 18.5-24.9	561 (42.7)	1.03 (0.56, 1.91)			0.77 (0.47, 1.27)
Overweight: = 25-29.9	338 (25.7)	1.08 (0.57, 2.04)			0.63 (0.38, 1.05)
Obesity: ≥30	356 (27.1)	0.76 (0.40, 1.43)			0.73 (0.44, 1.22)
Number of Vaginal Sex Acts in Past 3 Months					
Mean (Std Dev)	26.5 (24.7)	0.99 (0.98, 1.00)	N/A	N/A	N/A
Median (25th, 75th %tile)	20 (7,36)				
Received Money, Material Goods, Gifts, Drugs, or Shelter in Exchange for Vaginal or Anal Sex in the Past Year[4]					
No	1230 (93.7)	Ref	Ref	N/A	N/A
Yes	73 (5.6)	0.70 (0.47, 1.06)	0.79 (0.56, 1.13)		
Any Sexually Transmitted Infection[5]					
No (All Negative)	1028 (78.3)	N/A	N/A	N/A	N/A
Yes (Any positive)	285 (21.7)				
Chlamydia					
Negative	1138 (86.7)	N/A	Ref	N/A	N/A
Positive	175 (13.3)		0.85 (0.69, 1.05)		
Gonorrhea					
Negative	1255 (95.6)	N/A	N/A	N/A	N/A
Positive	58 (4.4)				
Trichomoniasis[4]					
Negative	1221 (93.0)	N/A	N/A	N/A	N/A
Positive	91 (6.9)				
Syphilis					
Negative	1297 (98.8)	N/A	Ref	N/A	N/A
Positive	16 (1.2)		1.30 (0.77, 2.19)		
Worried about Having Ring Inside Her Every Day for At Least A Year					
Not at all worried	926 (70.5)	Ref	Ref	N/A	N/A
Somewhat worried	366 (27.9)	0.72 (0.55, 0.92)	0.87 (0.72, 1.04)		
Very worried	21 (1.6)	0.77 (0.35, 1.70)	0.61 (0.33, 1.13)		
Time-dependent Characteristics					
Percentage of Vaginal Sex Acts Protected by a Condom in Past 7 Days					
0%	349 (26.6)	Ref	N/A	N/A	N/A
1-49%	66 (5.0)	1.11 (0.74, 1.66)			
50-99%	105 (8.0)	0.90 (0.64, 1.28)			
100% (or no sex)	793 (60.4)	1.16 (0.80, 1.70)			
Male or Female Condom Use During Last Act of Vaginal Sex in Past 7 Days[4]					
No Sex in Past 7 Days	315 (24.0)	Ref	Ref	N/A	Ref
Neither	390 (29.7)	0.98 (0.68, 1.43)	0.88 (0.78, 1.00)		0.88 (0.76, 1.03)
Male, Female or Both	602 (45.9)	0.89 (0.75, 1.06)	0.85 (0.76, 0.95)		0.95 (0.82, 1.10)
Anal Sex in Past 3 Months[4]					

No	1287 (98.0)	N/A	N/A	N/A	N/A
Yes	25 (1.9)				
Number of Male Sex Partners in Past 3 Months					
0-1	1102 (83.9)	N/A	N/A	N/A	N/A
2	156 (11.9)				
3 or more	55 (4.2)				
Sex with Primary Partner in Past 3 Months					
No	2 (0.2)	N/A	N/A	N/A	N/A
Yes	1305 (99.4)				
No Primary Partner	6 (0.5)				
Primary Partner Knows Participant is Taking Part in the Study					
No/not sure	331 (25.2)	Ref	Ref	Ref	N/A
Yes	976 (74.3)	1.47 (1.04, 2.06)	1.25 (0.99, 1.58)	1.16 (0.87, 1.54)	
No primary partner	6 (0.5)	1.00 (1.00, 1.00)	1.22 (0.85, 1.75)	1.96 (1.07, 3.60)	
Primary Partner Knows Participant is using Vaginal Ring					
No/Unsure	472 (36.0)	Ref	Ref	N/A	N/A
Yes	835 (63.6)	0.87 (0.63, 1.19)	0.87 (0.71, 1.07)		
No Primary Partner	6 (0.5)	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)		
Same Primary Partner in Past 3 Months					
No	45 (3.4)	Ref	N/A	N/A	Ref
Yes	1262 (96.1)	0.64 (0.43, 0.95)			1.43 (0.95, 2.15)
No primary partner	6 (0.5)	0.75 (0.42, 1.35)			1.73 (0.99, 3.04)
HIV-1 Status of Primary Partner in Past 3 Months					
Negative	707 (53.9)	Ref	N/A	N/A	N/A
Positive	22 (1.7)	0.70 (0.31, 1.60)			
Don't Know	578 (44.0)	1.10 (0.94, 1.29)			
No Primary Partner	6 (0.5)	1.00 (1.00, 1.00)			
Primary Partner is Taking Antiretroviral Drugs[4]					
No	1101 (83.9)	Ref	Ref	N/A	N/A
Yes	15 (1.1)	1.66 (0.58, 4.77)	1.29 (0.79, 2.12)		
Don't know	190 (14.5)	1.16 (0.93, 1.46)	0.99 (0.87, 1.14)		
No Primary partner	6 (0.5)	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)		
Primary Partner is Circumcised[4]					
No	705 (53.7)	N/A	Ref	Ref	N/A
Yes	551 (42.0)		1.00 (0.87, 1.15)	0.88 (0.72, 1.09)	
Don't Know	51 (3.9)		1.06 (0.75, 1.49)	1.27 (0.83, 1.94)	
No Primary Partner	6 (0.5)		1.00 (1.00, 1.00)	1.00 (1.00, 1.00)	
Bothered Wearing Ring Every Day[6,7]					
No		N/A	N/A	N/A	N/A
Yes					
Number of Times Started or Had Her Menstrual Period in the Last 3 Months[7]					
0		Ref	Ref	N/A	Ref
1		0.92 (0.75, 1.13)	0.85 (0.71, 1.01)		0.92 (0.76, 1.12)

2		1.01 (0.80, 1.27)	0.94 (0.79, 1.11)		0.79 (0.63, 0.98)
3		1.20 (0.92, 1.55)	0.91 (0.78, 1.07)		0.75 (0.60, 0.95)
Used Something[8] to Control Spotting or Bleeding in Last 3 Months[4,9]					
No	7 (0.5)	N/A	Ref	N/A	Ref
No Spotting/Bleeding	295 (22.5)		0.94 (0.60, 1.48)		0.92 (0.48, 1.77)
Yes	1008 (76.8)		0.76 (0.48, 1.19)		0.74 (0.38, 1.42)
Put Anything Inside Vagina in Last 3 Months[4,9]					
No	518 (39.5)	Ref	Ref	Ref	Ref
Yes	793 (60.4)	0.86 (0.74, 1.00)	0.90 (0.81, 1.01)	0.83 (0.71, 0.97)	0.94 (0.82, 1.09)
Primary Family Planning Method[10]					
Implants	255 (19.4)	1.64 (1.15, 2.34)	1.40 (1.10, 1.77)	1.61 (1.17, 2.21)	1.59 (1.21, 2.09)
Intrauterine device	161 (12.3)	1.21 (0.85, 1.71)	0.88 (0.70, 1.10)	1.40 (1.00, 1.98)	0.77 (0.57, 1.05)
Norethisterone enanthate	200 (15.2)	1.45 (1.05, 2.01)	1.53 (1.18, 1.98)	1.12 (0.80, 1.56)	1.47 (1.06, 2.03)
Depot medroxyprogesterone acetate	515 (39.2)	1.47 (1.10, 1.97)	1.13 (0.91, 1.40)	1.67 (1.25, 2.22)	1.95 (1.50, 2.55)
Other (includes oral contraceptive pills, sterilization, male or female condoms only, no family planning method)[11]	182 (13.9)	Ref	Ref	Ref	Ref

[1] Four separate multivariable models are shown. Site (not shown), age, study time, and calendar time were included in all models and additional covariates included were based on model selection criteria. Note that the number of participant-visits varies due to missing data longitudinally for the selected covariates in a given model.
[2] Covariates with a 95% confidence interval (CI) lower bound >1.00 are highlighted in dark green, and those with a 95% CI upper bound <1.00 are highlighted in dark orange. We also flagged covariates as having a "marginal" positive and negative association with adherence having a 95% CI upper bound ≥1.00 and ≤1.10 (light green), and a 95% CI lower bound ≥0.90 and ≤1.00 (light orange), respectively. Given our desire to broadly identify participant-characteristics that may be important for future research in this study cohort, we identified "marginal" associations recognizing that we may have been under-powered to detect a statistically significant association.
[3] N/A indicates that the characteristic was evaluated in univariable models but was not selected into the multivariable model.
[4] Category with missing data at baseline N (%): Alcoholic Drinks Per Week: 1 (0.1%), Body Mass Index: 17 (1.3%), Anal Sex in Past 3 Months: 1 (0.1%), Male or Female Condom Use During Last Act of Vaginal Sex in Past 7 Days: 6 (0.5%), Primary Partner is Taking Antiretroviral Drugs: 1 (0.1%), Used Something to Control Spotting or Bleeding in Last 3 Months: 3 (0.2%), Put Anything Inside Vagina in Last 3 Months: 2 (0.2%), Received Money, Material Goods, Gifts, Drugs, or Shelter in Exchange for Vaginal or Anal Sex in the Past Year: 10 (0.8%), Trichomoniasis: 1 (0.1%).
[5] Includes baseline results only for chlamydia, gonorrhea, trichomoniasis and syphilis.
[6] This variable is missing if a participant did not have a ring in her possession during the visit-month.
[7] No data was collected at baseline.
[8] Includes tissue, toilet paper, cloth or cotton wool put inside the vagina, or tissue, toilet paper, cloth or cotton wool placed in underwear/clothing, or tampon alone, or sanitary pad alone, or water without soap inside the vagina, or water with soap inside the vagina, or anything else.
[9] Missing data were imputed using the convention of last value carried forward, however, some missing values could have remained if all quarterly data was missing.
[10] Family planning method considered to be the primary one in use at the visit.
[11] At baseline, frequency of use is as follows: oral contraceptive pills [N=137 (10.4%)], sterilization (tubal ligation/hysterectomy/laparoscopy/other surgical procedure that causes sterilization) [N=45 (3.4%)], male or female condoms only (N=0), no family planning method (N=0).

Chapter 5. CONCLUSIONS

In this dissertation utilizing data from the MTN-020/ASPIRE study, our goal was to examine the impact of adherence on various aspects of a large-scale HIV-1 prevention study. Specifically, we were interested in assessing how effectively a real-time adherence monitoring program improved adherence while maintaining the study blind over follow-up, on how advanced analytical methods that consider varying levels of adherence impact efficacy estimates of the dapivirine ring against HIV-1 infection, and on whether correlates for adherence could be identified for various markers representing recent and continuous use of the product. Each aim lends evidence to the importance of incorporating valid adherence markers into the design and implementation of a large-scale HIV-1 prevention trial with user-defined PrEP agents such as the dapivirine ring.

The first aim of this dissertation describes the design and implementation of a real-time adherence monitoring program. The goal was to assess the feasibility and effectiveness of implementing such a strategy to provide feedback to investigators about product use among study participants while preserving the study blind for participants and investigators. This approach was considered to be novel for large-scale HIV-1 prevention studies, and it was shown to be successful as evidenced by our finding of a statistically significant increasing trend from 63% to 84% in adherence over calendar time. Our hypothesis stating that the shipping, testing and analysis of dapivirine levels in plasma would be completed and communicated to study sites on at least a quarterly basis from commencement of the real-time adherence monitoring program was shown to be true. Thus, we consider the additional efforts made to provide blinded, real-time information about participants' use or non-use of the vaginal ring to site investigators an effective means in safeguarding the primary endpoint analyses in so far as it aided in improving adherence to product use.

We also found that efforts to improve the use of the dapivirine ring during the implementation of a large-scale clinical study are not only possible, but critical to motivating participants to use an investigational product, despite participant perceptions of uncertainties regarding efficacy. In some studies of the same ring, younger women were found to be less adherent, partly due to their uncertainty about ring efficacy.[48,69] It is understandable that in clinical trial settings with participant-defined use of study product that there will be some participants who question the safety and efficacy of the product under investigation and therefore will also be reluctant to adhere to protocol requirements. Therefore, incorporating the most cost-effective and valid measure of adherence into study designs upfront is paramount to obtaining unbiased efficacy estimates, a pre-requisite to the ultimate aim of obtaining licensure for PrEP products.

The second aim of this dissertation evaluates the efficacy of a vaginal ring containing dapivirine compared to a placebo ring accounting for varying levels of adherence to product use. Our hypothesis that the estimated relative hazard of HIV-1 infection associated with dapivirine would be lowest among women who were more-fully adherent compared to those who were less-fully adherent. We recognized that intention-to-treat analyses, in the presence of imperfect adherence, were limited to assessing a combined effect of adherers and non-adherers. In the per-protocol analysis, where censoring of participants occurred at times when exposure to the vaginal ring stopped for an extended period of time, our estimates were only slightly greater than the intention to treat analyses (30% vs. 27%). In an as-treated analysis where data for participants were censored 30 days after their last reported dose of an oral PrEP agent among heterosexual men and women in Botswana, investigators found a rise in efficacy of 16%.[20] In another study of an oral PrEP agent conducted in Thailand among male and female injection drug users, an analysis restricted to

participants directly observed to have taken the study drug at least 5 days per week with no more than 2 consecutive days off study, efficacy estimates increased 7%.[3] Our approach did not, however, consider explicit use of the product and thus likely did not take into effect selection bias. Consequently, this may have been the reason for why per-protocol estimates were close to the intention-to-treat estimate. The alternative analytical approaches we employed aimed to provide unbiased estimates of efficacy in the context of incomplete follow-up and/or non-compliance to product use.

In principal stratification analyses our goal was to create strata that adjusted for personal characteristics reflected in the adherence variable, as an instrumental variable, that could have introduced post-randomization selection bias in estimating causal effects.[85,86] We created groups of participants based on high levels of predicted probabilities of adherence, however, instead of estimating adherence at 100%, as would be expected from the application of this method for a one-time, non-user-dependent intervention such as a one-dose vaccine. We assumed that the dapivirine arm model to define predicted probabilities of adherence could be appropriately applied to the placebo arm. Nevertheless, using the more stringent cutoff for adherence of >4.0 mg drug release, the distribution of predicted probabilities did not extend to the levels we were interested in (0.86 to 1.00), and thus HIV-1 efficacy models were inestimable. However, for the ≥0.9 mg cutoff we were able to adequately categorize women into high predicted probability of adherence groups. Despite the likelihood for misclassification of the adherence variable, we found HIV-1 efficacy increasing from 30% in the lower group to 54% in the highest group, nearly a doubling. Moreover, empirical estimates of HIV-1 efficacy trended similarly, and were contained within some of the confidence intervals of the predicted estimates as was shown in Figure 3.8.1. These findings

provide evidence that the effect of post-randomization selection bias was accounted for providing inference of causation within principal strata.[21] However, the precision of the efficacy estimates should be considered. It is evident by observing the wider width of the 95% CIs compared to the empirical estimates and their corresponding 95% CIs, that the estimates obtained by principal stratification contain a great deal more uncertainty. We attribute the additional uncertainty in estimates to variation due to model prediction and variation of the adherence measurement itself. The first factor is always present in statistical modeling, whereas the second is considered unavoidable in the ASPIRE dataset due to the lack of a strong adherence marker that perfectly defines whether a participant was or was not adherent enough to be fully protected when exposed to HIV-1. For marginal structural models, however, we found that depending on whether only the IPTW representing adherence alone, or multiplied by the pregnancy and informative loss to follow-up IPCWs, HIV-1 efficacy estimates of the dapivirine ring were ≥2 times greater, ranging from 49% to 57%, compared to the intention-to-treat estimate. We acknowledge that for these marginal structural models there were departures from the assumptions of consistency, for example. This is likely due to the presence of misclassification bias since defining a perfect cut point, based on drug release to define sustained exposure to the ring that warrants protection, was unknown. Likewise, the exchangeability, or no unmeasured confounding, assumption may have been violated in these models. The effect of the violations of assumptions is one in which we would expect attenuated HIV-1 efficacy estimates from the "true" efficacy. We hypothesize that non-differential misclassification of the exposure variable of adherence is present, as would follow if the consistency assumption is violated. In the event that this is the case, we must also evaluate the outcome variable of HIV-1 infection in order to better understand the effect on the efficacy estimates. However, we believe we can be confident that there is little to no misclassification of

HIV-1 status among participants in the MTN-020/ASPIRE study based on previous analyses using the HIV-1 testing algorithm, quality assurance measures in place during the study and confirmation of HIV-1 resistance to non-nucleoside reverse transcriptase drugs such as dapivirine.[40]

For the third aim of this dissertation, we identified demographic and self-reported risk behavior correlates to adherence to the use of the microbicidal vaginal ring. By utilizing a three-step process for model building, and after adjustment for important known correlates of adherence such as study site, age, time on study and calendar time, we found that participant characteristics such as primary partner knowledge that the participant was taking part in the study, and use of long-acting contraceptive methods were most strongly associated with adherent use of the ring. Likewise, worries about ring use, condom use during sex, episodes of menstrual bleeding and vaginal washing were associated with non-adherence. By identifying these correlates to adherence, we have gained a broader understanding of the factors that led to varying use of the product during study conduct. Understanding the characteristics of individuals who do and do not adhere to product use within the context of a large-scale trial provides a means for aiding in recruitment efforts for future trials of similar products. For example, by conducting a study to evaluate the use of a proposed PrEP agent using an active control prior to designing a phase III efficacy trial may provide valuable data regarding potential correlates to adherence. Found correlates could then be utilized to aid in recruitment efforts, targeting participants with a high propensity to adhere, in the population of interest.

Addressing issues of adherence in this phase III clinical trial to evaluate the HIV-1 efficacy of the dapivirine vaginal ring proved to be critical to the success of the study. As implied by our findings

in Aim 1, without a real time adherence monitoring program, it is likely the MTN-020/ASPIRE trial HIV-1 efficacy estimates would not have reached statistical significance at the $p<0.05$ level, and thus, the dapivirine ring may not have moved forward for licensure. At the time of this writing, the International Partnership for Microbicides has submitted an application to the European Medicines Agency for licensure of the dapivirine ring in South Africa and are currently working on submission to the US Food and Drug Administration (FDA). As part of this submission dossier, the Ring Study, another phase III trial conducted in parallel with MTN-020/ASPIRE, showed similar results, both in intention-to-treat and adherence-adjusted analyses. The consistency of results from this study provide additional evidence for the need to incorporate adherence measurements, in a blinded fashion, throughout study follow-up. Notably, the decision to mount the real-time adherence monitoring program was novel and this effort was made at the outset when MTN-003/VOICE results revealed that self-reported measures of adherence were far lower in biological measures (considered to be the gold standard) compared to participant reports. The intention-to-treat efficacy estimate of oral Truvada® compared to placebo in MTN-003/VOICE was -4% (95% CI: -49%, 27%, p=0.81). For tenofovir products alone, such as oral tenofovir disoproxil, the efficacy estimate was -49% (95% CI: -129%, 3%, p=0.07) and for tenofovir gel was 15% (95% CI: -21%, 39%, p=0.37). In post-hoc case-cohort analyses using levels of tenofovir only in blood plasma, estimates were seen to increase for the tenofovir-only products: TDF/FTC: -25% (95% CI: -158%, 39%, p=0.54); Oral TDF: 34% (95% CI: -44%, 69%, p=0.30); TFV gel: 66% (95% CI: 13%, 87%, p=0.025). For tenofovir-only investigational products in this study, there was a large difference between the adherence-adjusted results and intention-to-treat efficacy estimates, even though adherence levels were low overall. Also, although participants reported high usage of products on behavioral questionnaires (self-reported adherence measures showed an

average of 90% for face-to-face interviews, and 88% for audio-computer assisted self-interview), it is clear that appropriate biological measures should instead be used to evaluate participant adherence to product use, given the discrepancy seen between the two measures. The products examined in the MTN-003/VOICE trial did not move forward to licensure due to low efficacy estimates across the arms. Although in the MTN-020/ASPIRE study levels of biological measures of dapivirine in plasma and drug released from rings were a far improvement from what was seen in the MTN-003/VOICE trial, there is still room for improvement.

Since large-scale efficacy studies for HIV-1 prevention are complex, expensive and require a great deal of human resources to implement, it behooves the scientific community to continue to critically examine how best to improve their implementation. We maintain that the place to begin is by first improving on the definition and measurement of biological measures for adherence. This requires a close examination of the PK profile of investigational products, and a thoughtful understanding about how best to capture data related to its use or non-use as it relates to exposure to HIV-1. For example, the plasma >95 pg/mL marker for adherence provided an indication of whether the ring may have been in place at least 8 hours prior to a study visit. However, it did not provide an indicator for whether the ring had been in place consistently over the monthly visit so as to be effective in preventing HIV-1 infection had the participant been exposed at any time. From this marker, it is impossible to evaluate. For the >200 pg/mL plasma marker for adherence, it was found to be stronger in defining consistent use of the ring. However, based on PK profiles, a woman could have had the ring in place for 24 hours prior to the study visit and her levels would have also been >200 pg/mL. So, again, this measure does not provide evidence of coverage over times when she may have been exposed to HIV-1. Likewise, for the ≥0.9 mg marker as a cutoff of residual drug released in a ring, we are able to rule out no or very little use of the ring over the

monthly visit, however, we cannot rule out that participants may have intermittently used the ring. For the >4.0 mg marker, likewise, we are able to rule out women who definitely did not use the ring, however, some women whose uptake of dapivirine was less over the monthly visit may not have been included as adherent by this definition even though the ring was in place at all times. Thus, these markers all carry some level of misclassification, and so are inherently imperfect for assessing consistent use and thus, use when HIV-1 exposure may have been present. Our post-hoc analyses using even the causal inference gold standard of statistical methodology relevant to datasets with non-compliance could not fully remedy this problem due to misclassification of the adherence variable. Likewise, when evaluating potential correlates to adherence, we used four biomarkers representing cut points for recent and continuous use of the ring since no one marker fully represented adherence behavior as it related to risk for HIV-1 infection over participants' follow-up. Therefore, it's clear that a new approach to evaluating and measuring adherence should be considered.

In recent years several approaches to remedying the problem of evaluating and measuring adherence in large-scale HIV-1 efficacy trials of PrEP agents have been evaluated. In one study among a subset of mostly women who participated in HIV-1 prevention studies, including MTN-020/ASPIRE, a screening tool was developed based on constructs to assess adherence propensity and monitoring.[87] These scales showed acceptable to very good internal reliability. To implement such a tool in trials would entail the use of an additional survey at screening and throughout follow-up, which although considered by investigators to be easy and inexpensive to administer, could also contribute to participant research burden. However, benefits of such a tool are that it could be developed in several languages, used on participants in both randomization arms, and complement

biomarkers for adherence. Therefore, it is feasible that a tool such as this could be used in a study of a vaginal ring or other user-defined strategy to aid in providing insight into misclassification of adherence biomarkers as was seen in our studies of the dapivirine ring.

Another potential approach to consider is the collection of data needed to assess prevention-effective adherence, defined as using a PrEP agent during times at which there is HIV-1 risk. In a study that examined whether the HIV-1 negative partner in serodiscordant couples in Kenya and Uganda used PrEP as a bridge to ART (i.e., until his/her partner had used ART for ≥6 months) enough to be considered prevention-effective, investigators found that >75% of participant-visits considered high for HIV-1 risk met the criteria.[88] The idea behind this concept is that PrEP use is not needed when HIV-1 risk is absent, and when HIV-1 risk is present, PrEP use needs to be very high in order to provide protection. The efficient use of PrEP is important to its roll-out in both low- and well-resourced settings given competing priorities of treatments and prevention efforts[89] so it could be argued that this concept should be applied to large-scale HIV-1 efficacy studies as well. If that were the case, it likely would involve combining real-time sexual risk behavior data with biomarker data, which would undoubtedly involve a high level of coordination between the site investigators and the data center. However, if it means providing accurate and timely assessment of adherence for times when the participant is at high risk for HIV-1 infection, the extra effort may indeed pay off for the success of the trial.

Alternatively, much work has been done in the field of wearable technologies for collecting longitudinal health measures continuously, such as for assessing metabolic, cardiovascular and gastrointestinal disorders. For example, for monitoring ambulatory diabetes, Abbot Laboratories

developed a continuous glucose monitoring semi-invasive device that allows for collection of glucose levels in real time. This is one example of many types of wearable technology that are becoming available for use in biomedical applications in the form of patches, wrist bands, smart watches, et cetera.[90] With new technologies such as wearables used for data collection of biomarkers of health, it is reasonable to believe that such devices could also be employed in large-scale HIV-1 prevention trials. For example, if participants were provided a patch that remained on the body over the duration of the visit window, or throughout follow-up, investigators could evaluate real-time information about whether the participant had adequate levels of a given analyte, or even a pregnancy prophylactic, that was measurable and safe. The analyte or pregnancy prophylactic would be included in the formulation of the investigational drug and placebo. Feedback directly to the participant could then be given about his/her levels of the analyte or pregnancy prophylactic to help improve adherence to the use of the investigational product.

A final consideration to remedy the issue of designing and implementing adherence-based measures for HIV-1 prevention studies is on the development of adherence-independent means of delivery of PrEP agents. For example, the delivery of PrEP agents via implants or long-acting injectables (LAIs) might replace the need for self-reported adherence or monitoring of an analyte or the interventional drug, as was done in MTN-020/ASPIRE. The advantage to using a LAI over a user-defined delivery form is that once received, over the duration of expected use, there would be no question about whether the drug had been delivered or not, unlike the vaginal ring where a participant can remove it at any time. However, there are still considerations that need to be made that can affect the use of the product. If the participant does not attend visits, she/he will not have the opportunity to receive the injection. Also, acceptability of the delivery form is important to

uptake. If LAIs are implemented in a study, the propensity of a participant to use it according to the protocol will likely depend on his/her acceptability of the delivery form. In the TRIO study, a mixed methods study on multipurpose prevention technologies, it was found that 62% of women preferred the use of injections, 27% preferred pills and 11% preferred a vaginal ring.[74] When comparing preferences of the ring versus LAIs among women who had prior experience with LAIs for contraception, preference for the ring was lower compared to LAIs [adjusted Relative Risk Ratio (aRRR): 0.38, 95% CI: 0.16–0.94]. Women who had prior experience using implants/IUDs, however, preferred using rings over LAIs (aRRR: 3.13, 95% CI: 1.31–7.48). Another factor relevant to choosing LAIs was whether the woman indicated frequency of product use as an important feature. Another study showed that LAIs provide low frequency of use, low user burden and correct administration by a clinician,[91] although found that 70% of women reported prior use of LAIs whereas only 42% reported current use. Women will switch contraceptive methods due to side effects, contextual factors and other reasons.[92,93] Similar results regarding preference for LAIs were seen in a US sample of gay and bisexual men. When asked about their preferences for PrEP, 46% preferred long-acting LAIs compared to 14% preferring oral pills, 22% whichever of the two were most effective, and 10% had no preference.[92] Side effects and long-term health were the factors most important to uptake of LAIs in this study. These studies support the notion that prior experience with the delivery form, especially among women using contraceptives, and other factors such as age and frequency of use, inform whether the delivery form is one in which participants will adhere. In other words, even though LAIs appear to be a gold standard with respect to adherence, there are still potential problems for adherence to their use.

To further the field of HIV-1 prevention and answer important scientific questions for which an efficacy study is designed, valid adherence measures should be considered essential in large-scale HIV-1 prevention efficacy studies. Through this thorough evaluation of adherence, we have emerged with a broader understanding of a framework for effectively achieving the goal of a successful trial for HIV-1 prevention strategies such as the dapivirine ring. It is our hope that with careful planning and evaluation of appropriate adherence markers used in HIV-1 prevention trials, their utility will improve current methods to obtain unbiased estimates of efficacy. This would in turn move PrEP products into licensure more quickly, and ultimately provide more HIV-1 prevention strategies to those in need globally.

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APPENDIX I – STUDIES OF EFFICACY OF PRE-EXPOSURE PROPHYLACTIC AGENTS ACCOUNTING FOR ADHERENCE

Study Name	Location	Study Population	PrEP Agent(s) & Design	N	Adherence Definition	Estimated Adherence	MITT[1] HIV-1 Effectiveness Estimates (95% CI)	Adherence-adjusted Effectiveness Estimates (95% CI)	Ref
CAPRISA 004	South Africa	Women (18-40 years old)	1:1 randomization to either 1% TFV[2] gel (coitally-associated use - before and after sex, no more than 2 doses in 24 hours) or placebo gel	889	Estimated proportion sex acts covered by two gel doses, calculated per woman: 1/2 X (# returned used applicators each month/# reported sex acts that month). Applicators not returned considered not used. Median of each woman's monthly adherence estimates used as overall gel adherence measure. Adherence groups based on >80%, 50%-80% and <50%.	181,340 applicators dispensed, 95.2% returned. On Average 72.2% (median=60.2%) self-reported sex acts in the last 30 days were covered by 2 doses of gel. 38% with >80% gel adherence, 20.5% with 50%-80% gel adherence, and 41.5% with <50% gel adherence.	39% (6%, 60%), p=0.017	High Adherers (>80% gel adherence): 54% (4%, 80%), p=0.025; Intermediate Adherers (50%-80% gel adherence): 38% (-67%, 77%), p=0.343; Low Adherers (<50% gel adherence): 28% (-40%, 64%), p=0.303	Karim et al[12]
iPREX	Brazil, Ecuador, Peru, South Africa, Thailand, United States	Men who have sex with men & transgender women (18-67 years old)	1:1 randomization to either daily oral FTC/TDF[3] or placebo pill	2,499	Self-reported measures of pill use, returned pill counts and drug levels in a subgroup of HIV+ and matched with 2 controls, one from each study group selected among sero-negative subjects. Plasma was tested	Self-reported: FTC-TDF vs. placebo, respectively: Week 4 (mean: 89% vs. 92%, p<0.001), Week 8 (mean: 93% vs. 94%, p=0.006), after Week 8 (mean, 95% in both groups). Returned pills: 89% - 95% depending on	44% (15%, 63%), p=0.005	Pill use <50%: 32% (-41%, 67%), Pill use >= 50%: 50% (18%, 70%), p=0.48 between <50% and >= 50% groups; Pill use <90%: 21% (-31%,	Grant et al[13]

					for FTC & TFV and peripheral blood mononuclear cells (PBMC) were tested for FTC triphosphate (FTC-TP) and TFV diphosphate (TFV-DP).	whether counted unreturned bottles as not used or used, respectively. Drug levels in subgroup: No drug detected in plasma or cell specimens in the placebo ppts., 3/34 (9%) HIV+ ppts. had either FTC-TP or TFV-DP detected, 8% and 54% of HIV+ and control ppts., respectively, considered "on treatment" >50% of days had drug detected in plasma or PBMC. 51% overall for HIV- ppts.		52%), Pill use >= 90%: 73% (41%, 88%), p=0.02 between <90% and >=90% groups; Detectable drug level in blood: 92% (40%, 99%), p<0.001. After adjustment for reported unprotected receptive anal intercourse: 95% (70%, 99%), p<0.001.	
FEM-PrEP	Kenya, South Africa, Tanzania	Women (18-35 years old)	1:1 randomization to daily oral FTC/TDF or placebo	2,120	Self-reported measures of adherence, returned pill counts and drug levels in plasma of FTC & TFV.	Self-reported at time of study drug discontinuation: 95% of ppts. reported that they usually or always took assigned drug. Pill count: 88% of pill use on days when pills were available to ppts. Drug levels: considered 10 ng/mL of TFV as evidence that had taken drug in previous 48 hours. HIV+ ppts. in TDF/FTC group, 10 ng/mL in 7/27 (26%) at beginning of infection window, in 7/33 (21%) at the end of the window and 4/27 (15%) at both	6% (-52%, 41%), p=0.81	Availability of study drug after randomization: >80%: 25% (-30%, 56%), p=0.31; <= 80%: -96% (-517%, 38%), p=0.25. Note that study was stopped for futility by the Data Safety and Monitoring Board	Van Damme et al[14]

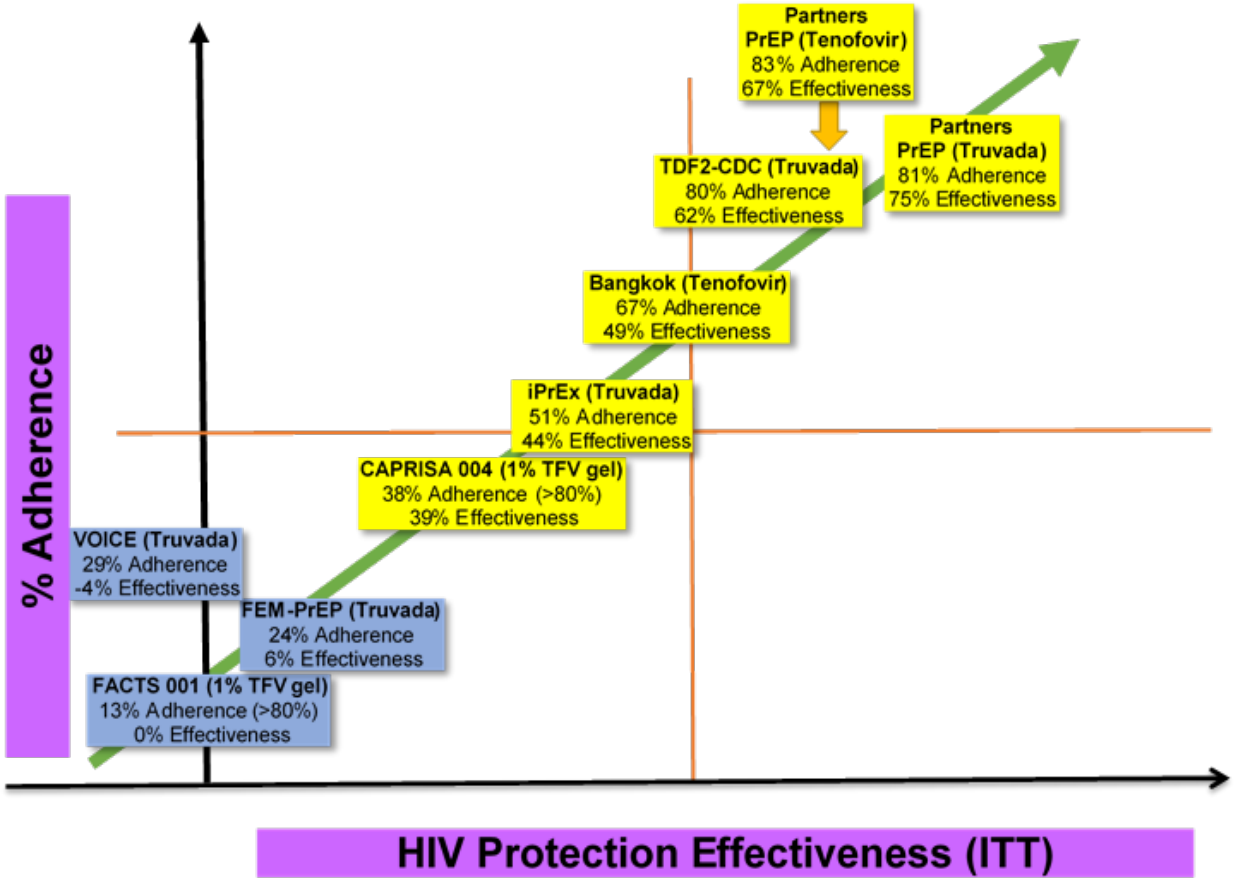
						visits. Among HIV- controls, 27/78 (35%) at beginning of infection window, 35/95 (37%) at the end of window and 19/78 (24%) at both visits.			
Partners PrEP Study	Kenya, Uganda	Heterosexual men and women with known HIV+ partners (serodiscordant couples) (18-65 years old)	1:1:1 randomization to daily oral TDF, FTC/TDF, or placebo	4,747	Monthly counts of the returned study bottles/pills, and plasma drug levels in a subgroup (case-cohort) of HIV+ (cases) and randomly selected HIV- ppts. (cohort). Also, adherence sub-study used unannounced pill counts and Medication Event Monitoring System (MEMS).	Pill count: 98% of dispensed study product was returned, 97% dispensed study product taken. Also, estimated 92.1% of total follow-up time when study medication was in use, accounting for missed visits, all reasons for non-dispensation of study medication, and non-adherence to dispensed study pills. Sub-study: Unannounced pill counts: median 99%, MEMS: median 97%.	TDF: 67% (44%, 81%), p<0.001; TDF/FTC: 75% (55%, 87%), p<0.001; By gender of HIV- partner: TDF: Male: 63% (20%, 83%), p=0.01; Female: 71% (37%, 87%), p=0.002; TDF/FTC: Male: 84% (54%, 94%), p<0.001; Female: 66% (28%, 84%), p=0.005.	In ppts. with any detectable tenofovir levels, TDF: 86% (57%, 95%), p <0.001; TDF/FTC: 90% (56%, 98%), p= 0.002. Among ppts. in the Adherence Sub-study: 100% (84%, 100%), p<0.001.	Baeten et al[15], Haberer et al[4]
TDF2-CDC Study	Botswana	Heterosexual men and women (18-35 years old)	1:1 randomization to daily oral FTC/TDF or placebo	1,219	Self-reported measures of adherence, returned pill counts and drug levels in plasma of FTC & TFV among HIV+ ppts and randomly selected HIV- TDF/FTC arm ppts. matched for sex and study site. Limited testing to ppts. who reported	Self-reported measure for preceding 3 days: 94.4% and 94.1% in TDF/TFC and placebo groups, respectively. P=0.32 between groups. Pill count: 84.1% and 83.7%, respectively. P=0.79 between groups. Drug levels in subgroup: 2/4	62% (22%, 83%), p=0.03; Males: 80% (25%, 97%), p=0.026; Females: 49% (-22%, 81%), p=0.107.	In as-treated analysis, where data for participants was censored 30 days after their last reported dose of study medication: 78% (41%, 94%), p=0.01. Males: 82% (-	Thigpen et al[16]

					having taken study medication within the previous 30 days.	(50%) HIV+ ppts. in TDF/FTC arm had detectable levels of tenofovir and emtricitabine in plasma at visit before and closest to estimated seroconversion date. In matched HIV- group, 55/69 (80%) and 56/56 (81%) had detectable levels of TDF and FTC, respectively.		3%, 99%), 0.065; Females: 76% (24%, 94%), p=0.021.	
VOICE	South Africa, Uganda, Zimbabwe	Women (18-45 years old)	1:1:1:1:1 randomization to daily oral TDF, FTC/TDF, placebo pill, TFV or placebo gel	5,029 (2,010 in TFV gel or gel placebo arms, and 3,019 in TDF, TDF/FTC or pill placebo arms)	Self-reported measures of adherence through questionnaires administered by interview monthly, monthly in-clinic counts of returned pills, empty pill bottles, or unused vaginal applicators (estimated adherence proportion = # product not returned/# days since the previous visit at which study products were dispensed); and quarterly audio-computer assisted self-interviews (ACASI). Plasma TFV levels and TFV and FTC levels from cervicovaginal fluid were measured in a subgroup (case-cohort).	Self-reported adherence measures: average - 90% for face-to-face interviews and 88% for ACASI measure. In ACASI, 47% of ppts., rated their ability to use the product daily, as instructed, during the previous 4 weeks as "very good" or "excellent." Clinic-based pill count: 86%. In random cohort of the case-cohort subset, quarterly plasma samples with TFV detected - Oral TDF: 30%, Oral TDF/FTC: 29%, TFV gel: 25%. Not detected in any quarterly plasma sample - Oral TDF: 58%, Oral TDF/FTC: 50%, TFV gel: 57%. Vaginal swab	Oral TDF: -49% (-129%, 3%), p=0.07; Oral TDF/FTC: -4% (-49%, 27%), p=0.81; TFV gel: 15% (-21%, 39%), p=0.37	Case-cohort analysis using whether TFV in plasma was detected or not at the first quarterly visit - Oral TDF: 34% (-44%, 69%), p=0.30; TDF/FTC: -25% (-158%, 39%), p=0.54; TFV gel: 66% (13%, 87%), p=0.025. Also, analysis of association of any TFV detection in any of available swab samples: -3% (-125%, 53%)	Marrazzo et al[17]

						samples with TFV detected - TFV gel: 49%. Not detected in any quarterly vaginal swab sample - TFV gel: 41%.			
Bangkok Tenofovir Study	Thailand	Injection drug users men and women (20-60 years old)	1:1 randomization to daily oral TDF or placebo	2,413	Daily directly observed therapy (DOT), or monthly visits without DOT (but could switch monthly) where adherence was measured using a study drug diary. Plasma TFV levels measured in all HIV+ ppts. and HIV- participants at 4 of the 17 clinics at study exit and designed an unmatched case-control analysis in ppts. receiving TFV	Based on study drug diaries: study drug taken an average of 84% of days and did not differ b/t tx arms (p=0.16) or time on study (p=0.22). Ppts. on DOT an average of 87% of time, median adherence on DOT = 95% and non-DOT = 100%. TFV detected in 1/177 (1%) ppts. in placebo group, and 100/151 (66%) in TFV group. In case-control subgroup, detected TFV in plasma in 5/13 (39%) of HIV+ ppts., and 93/138 (67%) in HIV- ppts.	49% (10%, 72%), p=0.01; Males: 38% (-18%, 68%), p=0.15; Females: 79% (17%, 97%), p=0.03	Adherence-defined analysis restricted to DOT ppts. who took study drug at least 71% of days (approx. 5 days per week) with no more than 2 consecutive days off study drug: 56% (-19%, 86%), p=0.11. Post-hoc analysis removing 2 ppts. with no TFV in plasma: 74% (17%, 94%), p=0.03. Comparing those with and without detectable TFV in plasma: 70% (2%, 91%), p=0.04.	Choopanya et al[18]

FACTS 001	South Africa	Women (18-30 years old)	1:1 randomization to either 1% TFV gel (coitally-associated use - before and after sex, no more than 2 doses in 24 hours) or placebo gel	2,059	Return of unused and used gel applicators. Product adherence was defined as the proportion of self-reported sex acts covered by the gel. Also, nested case-cohort sub-study examined drug levels in quarterly cervicovaginal lavage (CVL) samples in HIV seroconverters vs. controls.	Based on applicator counts & self-reported number of sex acts, used the gel during an average of 50-60% of sex acts per month. 13% of the participants used gel in >80% of sex acts.	Incidence Rate Ratio: 1.0 (0.7, 1.4)	TFV gel effectiveness highest in women who reported product use in >72% of sex acts based on applicator returns: IRR 0.43 (0.09-1.61); however, this subgroup represented only 20% of participants. In the case-cohort sub-study, high TFV in CVL (not defined in abstract) was significantly associated with a reduction in HIV acquisition (HR: 0.52; 95% CI: 0.27-0.99; p=0.04).	Rees, et al[19]
[1] Modified Intention-to-treat: Defined as intention-to-treat analysis excluding any ppts. for whom after randomization HIV-1 infection was subsequently determined to have occurred at or prior to enrollment in the study.									
[2] 1% tenofovir in gel form formulated for topical cervicovaginal application.									
[3]TDF = tenofovir disoproxil fumarate, FTC = emtricitabine. TDF/FTC = Truvada. TDF was given at a dose of 300 mg and FTC was given at a dose of 200 mg. These are standard doses for treatment of HIV-1.									

APPENDIX II – RELATIONSHIP BETWEEN ADHERENCE AND HIV-1 PROTECTION IN PREP STUDIES



APPENDIX III – INCLUSION/EXCLUSION CRITERIA FROM THE MTN-020/ASPIRE PROTOCOL

Inclusion Criteria

Women must meet all of the following criteria to be eligible for inclusion in the study:

- Age 18 through 45 years (inclusive) at screening, verified per site SOPs; within this range, sites may restrict the upper age limit per site SOPs, to target women at high risk of HIV infection
- Able and willing to provide written informed consent to be screened for and to take part in the study
- Able and willing to provide adequate locator information, as defined in site SOPs
- HIV-uninfected based on testing performed by study staff at screening and enrollment (per applicable algorithm in Appendix II)
- Per participant report, sexually active, defined as having vaginal intercourse at least once in the 3 months prior to screening
- Using an effective method of contraception at enrollment, and intending to use an effective method for the duration of study participation; effective methods include hormonal methods (except contraceptive ring); intrauterine device (IUD); and sterilization (of participant, as defined in site SOPs)
- At screening and enrollment, agrees not to participate in other research studies involving drugs, medical devices, vaginal products, or vaccines for the duration of study participation
  Note: Tampons may be used for the duration of the trial.

Exclusion Criteria

Women who meet any of the following criteria will be excluded from the study:

Per participant report at screening:

- Intends to become pregnant during study participation
- Plans to relocate away from the study site during study participation
- Plans to travel away from the study site for more than 8 consecutive weeks during study participation
- Is pregnant
  Note: A documented negative pregnancy test performed by study staff is required for inclusion; however a self-reported pregnancy is adequate for exclusion from the study.
- Currently breastfeeding
- Diagnosed with urinary tract infection (UTI)
  Note: Otherwise eligible participants diagnosed with UTI during screening are offered treatment and may be enrolled after completing treatment and all symptoms have resolved.

If treatment is completed and symptoms have resolved within 28 days of obtaining informed consent for screening, the participant may be enrolled.

- Diagnosed with pelvic inflammatory disease, an STI or reproductive tract infection (RTI) requiring treatment per current WHO guidelines
  Note: Otherwise eligible participants diagnosed during screening with pelvic inflammatory disease or STI/RTI requiring treatment per WHO guidelines — other than asymptomatic BV and asymptomatic candidiasis — are offered treatment and may be enrolled after completing treatment and all symptoms have resolved. If treatment is completed and symptoms have resolved within 28 days of obtaining informed consent for screening, the participant may be enrolled. Genital warts requiring treatment also must be treated prior to enrollment. Genital warts requiring therapy are defined as those that cause undue burden or discomfort to the participant, including bulky size, unacceptable appearance, or physical discomfort.
- Has a clinically apparent Grade 2 or higher pelvic exam finding (observed by study staff) as per the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events Version 1.0, December 2004 (Clarification dated August 2009), Addendum 1-Female Genital Grading Table for Use in Microbicide Studies
  Note: Cervical bleeding associated with speculum insertion and/or specimen collection judged to be within the range of normal according to the clinical judgment of the Investigator of Record (IoR)/designee is considered expected non-menstrual bleeding and is not exclusionary.
  Note: Otherwise eligible participants with exclusionary pelvic exam findings may be enrolled/randomized after the findings have improved to a non-exclusionary severity grading or resolved. If improvement to a non-exclusionary grade or resolution is documented within 28 days of providing informed consent for screening, the participant may be enrolled.

Participant report and/or clinical evidence of any of the following:

- Known adverse reaction to any of the study products (ever)
- Known adverse reaction to latex (ever)
- Chronic vaginal candidiasis
- Non-therapeutic injection drug use in the 12 months prior to Screening
- Post-exposure prophylaxis (PEP) for HIV exposure within 6 months prior to enrollment
- Last pregnancy outcome 90 days or less prior to enrollment
- Gynecologic or genital procedure (e.g., tubal ligation, dilation and curettage, piercing) 90 days or less prior to enrollment
- Recent participation in any other research study involving drugs, medical devices, vaginal products, or vaccines, within 60 days of enrollment
- Participation in the MTN-003, Vaginal and Oral Interventions to Control the Epidemic (VOICE) clinical trial, or any other HIV prevention study using systemic or topical antiretroviral medications, within 12 months of enrollment
- As determined by the IoR/designee, any significant uncontrolled active or chronic cardiovascular, renal, liver, hematologic, neurologic, gastrointestinal, psychiatric, endocrine, respiratory, immunologic disorder or infectious disease, including active tuberculosis

Has any of the following laboratory abnormalities at Screening Visit:

- Aspartate aminotransferase (AST) or alanine transaminase (ALT) Grade 1 or higher as per the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events Version 1.0, December 2004 (Clarification dated August 2009)
- Creatinine Grade 2 or higher as per the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events Version 1.0, December 2004 (Clarification dated August 2009)
- Hemoglobin Grade 2 or higher as per the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events Version 1.0, December 2004 (Clarification dated August 2009)
- Platelet count Grade 1 or higher as per the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events Version 1.0, December 2004 (Clarification dated August 2009)
- Pap result Grade 2 or higher according to the Female Genital Grading Table for Use in Microbicide Studies Addendum 1 to the DAIDS Table for Grading Adult and Pediatric Adverse Events, Version 1.0, December 2004 (Clarification dated August 2009)
  Note: Otherwise eligible participants with an exclusionary test may be re-tested during the screening process.
  Note: Women with a documented normal result within the 12 months prior to enrollment need not have Pap smear during the screening period. Women with a Grade 1 abnormal Pap smear can be enrolled upon completion of the initial phase of evaluation if no current treatment is indicated (based on local standard of care for management of abnormal cervical cytology). Need for a repeat Pap within 6 months does not preclude enrollment prior to that result becoming available.
- Has any other condition that, in the opinion of the IoR/designee, would preclude informed consent, make study participation unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives.

APPENDIX IV – SCHEDULE OF STUDY VISITS AND EVALUATIONS FROM THE MTN-020/ASPIRE PROTOCOL

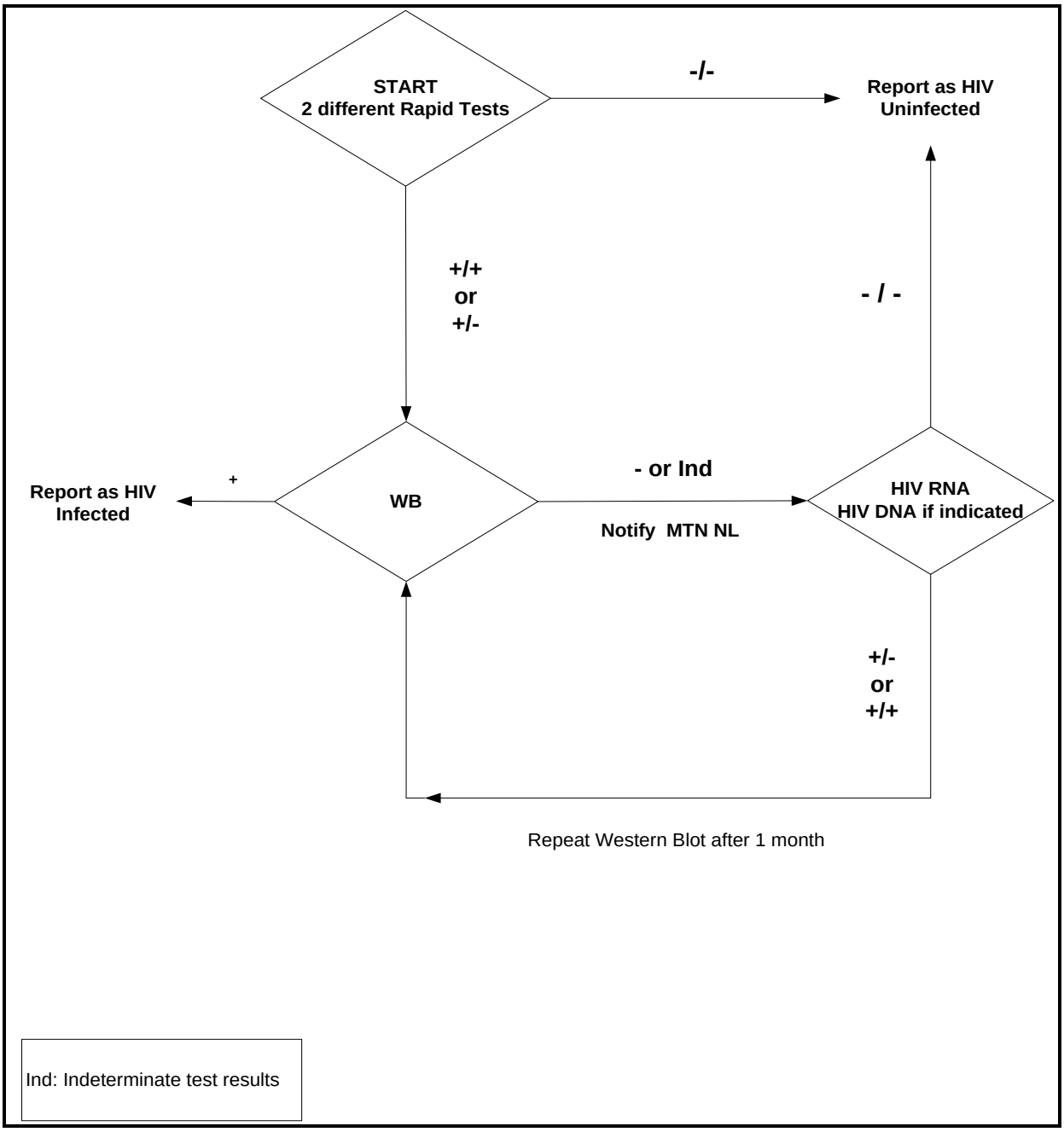
	SCR	ENR	Monthly Visits	Quarterly Visits	Semi-Annual Visits	PUEV	Study Exit/ Term. Visit
ADMINISTRATIVE AND REGULATORY							
Obtain informed consent	X	X					
Assign a unique Participant Identification (PTID) number	X						
Assess and/or confirm eligibility	X	X					
Collect/review/update locator information	X	X	X	X	X	X	X
Randomization		X					
Provide reimbursement	X	X	X	X	X	X	X
Schedule next visit	*	X	X	X	X	X	*
BEHAVIORAL							
Contraceptive counseling	X	X	X	X	X		
Protocol adherence, including vaginal ring adherence counseling		X	X	X	X		
HIV/STI risk reduction counseling	X	X	X	X	X	X	X
HIV pre- and post-test counseling	X	X	X	X	X	X	X
Conduct a behavioral assessment includes sexual activity, condom use, and intravaginal practices		X		X	X	X	X
Conduct an adherence assessment			X	X	X	X	
Conduct an acceptability assessment				X	X	X	
Conduct social harms assessment				X	X	X	
CLINICAL							
Obtain/update medical and menstrual history	X	X	X	X	X	X	X
Obtain/update concomitant medications	X	X	X	X	X	X	X
Conduct a physical examination	X	X	*	X	X	X	
Perform a pelvic exam	X	X	*	*	X	X	*
Prescribe contraceptives	*†	*†	*†	*†	*†	*†	*†
Disclose available test results		X	X	X	X	X	X
Record/update AEs			X	X	X	X	X
Treat or prescribe treatment for UTIs/RTIs/STIs or refer for other findings	*	*	*	*	*	*	*
LABORATORY							
URINE: hCG	X	X	X	X	X	X	X
URINE: Urine culture	*†	*†	*†	*†	*†	*†	*†
URINE: NAAT for GC/CT	X	*†	*†	*†	X	*†	*†

BLOOD	HIV-1 Serology	X	X	X	X	X	X	X
	CBC with platelets	X			X	X	X	
	Chemistries	X			X	X	X	
	Syphilis serology	X		*	*	*	X	
	Plasma		◊		X	X	X	X

		SCR	ENR	Monthly Visits	Quarterly Visits	Semi-Annual Visits	PUEV	Study Exit/ Term. Visit
PELVIC	Rapid test for Trichomonas	X	*†	*†	*†	X	*†	*†
	Herpes lesion testing	*†	*†	*†	*†	*†	*†	*†
	Vaginal fluid pH	*†	X	*†	*†	X	X	*†
	KOH wet mount for candidiasis	*†	*†	*†	*†	*†	*†	*†
	Saline wet mount for BV	*†	*†	*†	*†	*†	*†	*†
	Gram stain		X			X	X	
	Vaginal fluid			X	X	X	X	X
	Pap Smear interpretation	*					X	
	Endocervical swab		X			X	X	
STUDY PRODUCT/ SUPPLIES								
Provision of study specified condoms		X	X	X	X	X	X	X
Provision of study vaginal ring instructions			X	*	*	*		
Provision of one study vaginal ring for insertion			X	X	X	X		
Participant or clinician/designee to remove used study vaginal ring				X	X	X	X	
Digital exam(s) by clinician to check vaginal ring placement			X	₪ *	*	*		
Demonstrated attempt to remove and reinsert the ring			X					
Collection of used study vaginal ring				X	X	X	X	
Dispense a bottle of water, at select sites with capacity			*	*	*	*		

X mandatory, * If indicated, † per local standard of care, ◊= for archive, ₪= required at Month 1

APPENDIX V – MTN-020/ASPIRE HIV-1 TESTING ALGORITHM



VITA

In 1991, Marla J. Husnik received a Bachelor of Arts degree in Mathematics and Philosophy from St. Olaf College in Northfield, Minnesota. She received a Master of Science degree in Biostatistics from the University of Minnesota in 1995. From 1996 through 1998 she worked as an Implementation Analyst at the Health Outcomes Institute, and a Senior Epidemiologist at the Minnesota Department of Health. In 1999, Marla joined the Statistical Center for HIV/AIDS Research and Prevention (SCHARP) at the Fred Hutchinson Cancer Research Center (FHCRC) in Seattle, Washington as a Statistical Research Associate. While working at SCHARP-FHCRC, she contributed to multiple phase I-IIIb clinical trials sponsored by the National Institutes of Health – Division of AIDS that evaluated behavioral and microbicidal HIV prevention strategies, including the MTN-020/ASPIRE study evaluating the dapivirine vaginal ring. In 2012, Marla enrolled in the doctoral program in Epidemiology at the University of Washington concurrent with her role as a Statistical Manager at SCHARP-FHCRC. She contributed to the statistical aspects of the MTN-020/ASPIRE study, from which this dissertation pertains, throughout her doctoral training.