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Phase 1 Safety, Pharmacokinetics, and Pharmacodynamics of Dapivirine and Maraviroc Vaginal Rings: a Double-Blind Randomized Trial

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Drs. Chen, Panther, Marzinke, Hoesley, van der Straten, Soto-Torres, Nel, Dezzuti, Ms. Husnik, Ms. Johnson, and Ms. Rabe do not have any related conflicts of interest.

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Abstract:

Background

Variable adherence limits effectiveness of daily oral and intravaginal tenofovir-containing pre-exposure prophylaxis. Monthly vaginal antiretroviral rings are one approach to improve adherence and drug delivery.

Methods

MTN-013/IPM 026, a multi-site, double-blind, randomized, placebo-controlled trial in 48 HIV-negative U.S. women, evaluated vaginal rings containing dapivirine (25 mg) and maraviroc (100 mg), dapivirine-only, maraviroc-only, and placebo used continuously for 28 days. Safety was assessed by adverse events. Drug concentrations were quantified in plasma, cervicovaginal fluid (CVF), and cervical tissue. Cervical biopsy explants were challenged with HIV *ex vivo* to evaluate pharmacodynamics.

Results

There was no difference in related genitourinary adverse events between treatment arms compared to placebo. Dapivirine and maraviroc concentrations rose higher initially before falling more rapidly with the combination ring compared to relatively stable concentrations with the single drug rings. Dapivirine concentrations in CVF were 1 and 5 $\log_{10}$ greater than cervical tissue and plasma for both rings. Maraviroc was consistently detected only in CVF. Dapivirine and maraviroc CVF and dapivirine tissue concentrations dropped rapidly after ring removal. Cervical tissue showed a significant inverse linear relationship between HIV replication and dapivirine levels.

Conclusions

In this first study of a combination microbicide vaginal ring, all four rings were safe and well tolerated. Tissue dapivirine concentrations were 1,000 times greater than plasma concentrations and single drug rings had more stable pharmacokinetics. Dapivirine, but not maraviroc, demonstrated concentration-dependent inhibition of HIV-1 infection in cervical tissue. Since maraviroc concentrations were consistently detectable only in CVF and not in plasma, improved drug release of maraviroc rings is needed.

Key words: microbicide, pre-exposure prophylaxis, dapivirine, maraviroc, vaginal rings, *ex vivo* challenge assay

Trial registration: ClinicalTrials.gov identifier: NCT01363037

INTRODUCTION

Microbicides are topically-applied products designed to prevent sexual acquisition of HIV through pre-exposure prophylaxis (PrEP). Vaginal tenofovir gel was partially protective when used before and after intercourse. However, lower adherence was associated with higher rates of HIV acquisition.[1] Several daily oral and vaginal dosing PrEP trials also found low rates of adherence, which impacts effectiveness.[2,3]

Vaginal rings providing sustained drug release hold promise for increased adherence compared to products requiring daily dosing. Additionally, rings have the capacity to release multiple antiretrovirals (ARV), which may reduce the risk of acquisition of ARV-resistant HIV. Topical administration also provides the highest ARV concentrations in genital tissues, the site of transmission in sexually-acquired HIV.

Dapivirine (DPV), a non-nucleoside reverse transcriptase inhibitor (NNRTI), is under evaluation as a microbicide in vaginal ring, gel, and film formulations.[4-10] Two randomized controlled trials are testing the efficacy of DPV vaginal rings for PrEP.[11,12] Although DPV is not effective against HIV-2, the global incidence of HIV-2 sexual transmission is vastly lower compared to that of HIV-1.[13] As a drug class, NNRTIs are attractive candidates for the prevention of sexually transmitted HIV-1 due to high concentrations in the female genital mucosa when administered systemically.[14] However, the potential for development of resistance to this class is high, especially in cases of intermittent or noncompliant use.

The greatest amount of data regarding the effectiveness of NNRTIs for prevention of mucosal transmission of HIV-1 comes from studies of mother-to-child transmission (MTCT) where intrapartum administration of a single dose of nevirapine significantly decreased rates of MTCT of HIV-1.[15] Concerns about nevirapine resistance surfaced after follow-up studies of mother-infant pairs demonstrated treatment failures in those who began NNRTI-containing ARV therapy within 6 months postpartum, but this difference was insignificant if ARVs were started greater than 6 months postpartum.[16,17] Sexual transmission of ART-resistant HIV-1 is a known phenomenon, though its clinical significance remains in question.[18] It is not known whether sustained release of NNRTIs in high concentration at the site of transmission, such as in the form of drug-eluting vaginal rings, will decrease their effectiveness in the face of drug resistant HIV-1 transmission. Given the concern for NNRTI resistance with single-drug therapy, combining an NNRTI with another ARV holds promise for effectiveness against HIV transmission while reducing the risk of developing resistance.

Maraviroc (MVC), a CCR5 receptor antagonist approved as a second-line oral treatment for HIV, acts early in the HIV life cycle by blocking access to CCR5, making it an attractive microbicide candidate since CCR5-tropic HIV is preferentially transmitted in the genital tract. In addition, MVC is an effective entry inhibitor for both HIV-1 and HIV-2.[19] MVC is not active against viruses using co-receptors other than CCR5 thus is well suited for prevention in regions where the virus most likely to be transmitted primarily uses CCR5.[20-22] Since DPV is active against HIV regardless of viral co-receptor tropism, a combination of DPV and MVC has potential for complementary efficacy to prevent HIV acquisition.

This study evaluated the safety, pharmacokinetics (PK), and pharmacodynamics (PD) of vaginal rings containing DPV and MVC, DPV-only, and MVC-only compared to placebo.

METHODS

MTN-013/IPM 026, a Phase I, multi-site, double-blind, four-arm, randomized trial, evaluated vaginal rings containing 25 mg DPV plus 100 mg MVC, 25 mg DPV, 100 mg MVC, or placebo. Rings were used continuously for 28 days, followed by 24 days off product. The study was conducted at the University of Pittsburgh (Pittsburgh, PA), the University of Alabama at Birmingham (Birmingham, AL), and Fenway Institute (Boston, MA). All sites received institutional review board approval.

The vaginal ring was an off-white, flexible ring (56 mm outer diameter × 7.7 mm cross-sectional diameter) containing drug dispersed in a platinum-catalyzed-cured silicone matrix designed for sustained release of drug over at least 28 days. The placebo ring was manufactured with the same components, except it contained USP titanium dioxide as a colorant for blinding.

The primary objectives were to assess the safety of vaginal rings and the PK of DPV and MVC in cervicovaginal fluid (CVF), plasma and cervical tissue. Safety was evaluated as the proportion of women with related genitourinary adverse events (AEs) and proportion of women with any grade 2 or higher AEs, using the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events and Addendum 1 (Female Genital Grading Table for Use in Microbicide Studies).[23,24] A secondary objective was to evaluate adherence over 28 days of use. Exploratory objectives included evaluation of HIV inhibitory activity in cervical tissue obtained

on day 28 using the HIV *ex vivo* challenge assay, changes in the vaginal microenvironment, and presence of ring biofilms.

Eligible women were aged 18-40, HIV-negative, sexually abstinent, healthy, and using effective contraception. Major exclusion criteria included: pregnant or breastfeeding, CYP3A inducer and/or inhibitor use; chronic or recurrent candidiasis; significant blood chemistry or hematology abnormalities; sexually transmitted infection requiring treatment; clinically apparent gynecological abnormalities; or severe pelvic organ prolapse. Three months after recruitment began, eligibility criteria were revised per the Food and Drug Administration's request to exclude women infected with hepatitis B or C and/or decreased white blood cell counts less than $2000/mm^3$. Women enrolled at time of the modification were tested for hepatitis B and C; none were infected.

After providing written informed consent and undergoing screening evaluations, eligible participants were randomized in a 1:1:1:1 ratio to a vaginal ring, stratified by clinical site. They self-inserted the ring, followed by a pelvic exam performed by a clinician to check ring placement. Blood was drawn at 0, 1, 2, 4, and 6 hours post-insertion for PK. Vaginal fluid was obtained for pH, Gram stain, and quantitative vaginal culture. CVF for PK was obtained using tear test strips.

Blood and CVF were collected for PK at a single time point on days 1, 2, 3, 5, 7, 14, and 21. A visual inspection of the vagina and cervix was performed during all pelvic exams to assess for epithelial changes.[25] On day 28, rings were removed and blood was drawn at 0, 1, 2, 4, and 6

hours post-removal for PK. CVF for PK and safety laboratory blood tests were obtained. Cervical tissue biopsies were collected for PK and PD (*ex vivo* HIV challenge of tissue) immediately after ring removal. For PK testing, the biopsies were weighed, immediately snap frozen, and stored at -80°C. A subset of 16 rings from Pittsburgh was assessed for biofilm formation with electron microscopy; the remaining 32 rings had residual drug levels quantified and used as a general measure of adherence. Adherence to ring use, defined as ring always in the vagina, was also assessed using Computer-Assisted Self Interviewing (CASI) weekly and by case report forms at each follow-up visit.

Participants returned on days 29, 30, 31, 35, 42, and 52 for blood for PK. Participants were randomized in a 1:1:1 ratio to CVF and cervical tissue collection for PK on day 31, 35, or 42. CVF was also obtained at day 52.

The sample size of 12 women per group was similar to other Phase 1 studies of vaginal microbicides. Randomization lists were generated for each site by the Statistical Data Management Center, with two permutated blocks of size 8 (two per arm) for assignment to each arm. Study sites received sequentially numbered, opaque, sealed envelopes containing prescriptions printed with the corresponding randomization number. Vaginal rings were supplied in identical overwrappers. Study staff, participants, and pharmacists were blinded to the random assignments of all participants.

DPV and MVC concentrations in plasma and CVF were quantified via validated ultra performance liquid chromatographic-tandem mass spectrometric (LC-MS/MS) methods as

previously described.[26-28] The lower limits of quantification (LLOQ) for DPV in plasma and CVF were 20 pg/mL and 0.05 ng/tear strip, respectively. The LLOQ for MVC in plasma and CVF were 0.5 ng/mL and 0.025 ng/tear strip, respectively. Tissue DPV and MVC quantification was performed using calibrators prepared in human plasma and matrix-specific tissue quality control samples. Following homogenization and protein precipitation, tissue DPV and MVC concentrations were determined via LC-MS/MS with LLOQ of 0.05 ng/sample and 0.20 ng/sample (both approximately 1 ng/mg based on typical biopsy weights), respectively.

For residual drug analysis, rings were cut into four pieces, combined in a bottle with 100 mL of acetone, and agitated for 24 hours at 180 rpm. An aliquot was removed, evaporated to dryness under nitrogen, reconstituted with acetonitrile, mixed, and centrifuged. An aliquot of supernatant was transferred to a 10 mL volumetric flask and diluted to volume with water. Samples were analyzed by isocratic reversed phase high performance liquid chromatography with ultraviolet detection at 210 nm.

Demographic and adherence data were analyzed using descriptive statistics. For the primary AE endpoints, each treatment arm was compared with placebo using Fisher's exact test. Descriptive statistics (median, interquartile ranges [IQR]) were used to summarize non-compartmental PK parameters, including area under the concentration-time curve to infinity (AUC_{inf}), time to peak concentration (T_{max}), peak concentration (C_{max}), and day 28 concentration (C_{28D}) at time of ring removal. PK parameters specific to each drug were compared among anatomical sites sampled and between study arms using Wilcoxon-Mann-Whitney rank sum tests.

Fresh cervical tissue samples from Pittsburgh were used for the *ex vivo* challenge assay.[29] Cervical biopsies were transported to the laboratory within 30 minutes and exposed to HIV-1_{BaL} for two hours, washed and placed in culture. Supernatant was collected and replenished on days 4, 7, 11, 14, and 17 of culture. On day 21, the biopsies were removed from culture, weighed, and placed in paraformaldehyde. HIV-1 replication was monitored in the supernatant using a p24gag ELISA (Alliance, Perkin-Elmer, Waltham, MA). Data from participants using the DPV-only, MVC-only and DPV/MVC rings were combined for the PK/PD analysis. DPV and MVC (ng/mL) detected concentrations at Day 28 were $\log_{10}$ transformed. Non-detected DPV and MVC (ng/mL) were imputed at ½ the median tissue weight LLOQ of 50 and 200 for DPV and MVC, respectively. The p24 at Days 4, 7, and 11 of the *ex vivo* assay were used in a linear, least squares regression, with subject as a covariate factor, to model the PK endpoints at Day 28, where a negative slope would support the finding of drug-mediated virus suppression. *Ex vivo* samples that were acellular on histology were excluded from analysis.

Vaginal swabs were collected from all participants at baseline and on days 7, 28, and 52, and cultured for aerobic and anaerobic bacteria. Nugent scores were assessed at baseline and on days 3, 28, 31, and 52. Modified Poisson regression and generalized estimating equations were used to assess the effect of ring use on prevalence of vaginal microflora and Gram stain Nugent score. Quantity of biofilm was measured by a semi-quantitative assessment of scanning electron microscopy photographs at 25× magnification.

RESULTS

The study was conducted between September 2011 and September 2012, and enrolled 48 women from Pittsburgh (n=24), Birmingham (n=16), and Boston (n=8). Participant characteristics by study arm (Table 1) and study flow (Figure 1) are presented. Forty-seven participants (98%) completed the study. One participant withdrew for personal reasons after day 29; data obtained prior to withdrawal were analyzed.

A total of 33 grade 1 and one grade 2 related genitourinary AEs were observed in 22 women. There were no statistically significant differences in the number of participants with related genitourinary AEs or any grade 2 or higher AEs between the placebo arm and any other treatment arms. Only two grade 2 AEs, migraine and metrorrhagia, were related to study product, both in the MVC arm. All three grade 3 AEs were unrelated (hypertension [DPV/MVC], migraine [MVC], and headache [MVC]). One grade 4 unrelated AE occurred in the MVC arm in a woman with a known sulfa hypersensitivity and an inadvertent sulfa exposure.

Plasma DPV concentrations in the DPV-only arm rose rapidly to achieve a broad steady-state plateau from day 5 through day 28 with late and highly variable median T_{max} 421 hours [IQR 464 hours] and C_{max} 231 [46] pg/mL (Table 2). This was followed by a fall in concentration after ring removal with terminal decay half-life of 76 [41] hours. The 6-hour and 24-hour plasma DPV concentrations achieved 41% (p=0.002) and 62% (p=0.02) of the overall plateau concentrations. By contrast, plasma DPV concentration with the DPV/MVC ring achieved a T_{max} 35 [41] hours after ring placement (C_{max} 294 [144] pg/mL) followed by a slow decline over 2 weeks then a plateau in the final 2 weeks (C_{28D} 88 [47] pg/mL). This was followed by a 94 [56] hour terminal half-life after ring removal. The plasma AUC_{inf} and C_{28D} were significantly higher with the DPV

ring compared to the DPV/MVC ring (p=0.01 and p=0.005, respectively). The plasma C_{max} trended lower with the DPV ring compared to the DPV/MVC ring (p=0.08).

The temporal pattern of CVF DPV concentration followed a similar pattern with a broad concentration plateau with the DPV ring (T_{max} 76 [582] hours) in contrast to a relatively earlier peaking (T_{max} 37 [49] hours, p=0.05) and gradually declining pattern with the DPV/MVC ring (Figure 2A). The CVF C_{max} was lower with the DPV ring compared to the DPV/MVC ring (Table 2, p=0.002). CVF AUC_{inf} also trended lower (p=0.08). In the week after ring removal, the CVF DPV concentrations fell to undetectable concentrations in all but one participant in each arm and were below detection in all participants from day 42 onward.

Day 28 tissue DPV concentrations were similar among DPV and DPV/MVC ring users (Table 2). Tissue DPV concentrations were quantifiable in all DPV users and 11 of 12 (92%) DPV/MVC users. Tissue DPV concentrations were roughly 4 $\log_{10}$ greater than plasma and 1 $\log_{10}$ lower than CVF concentrations. Tissue DPV concentrations dropped rapidly after ring removal, and were below LLOQ by day 31.

Plasma MVC concentrations were above LLOQ for two MVC ring users (0.62 ng/mL, 0.62 ng/mL) and two DPV/MVC users (0.51 ng/mL, 8.34 ng/mL), each for a single time point. Plasma MVC concentrations were below LLOQ in both rings at day 28. The CVF MVC concentration peaked on day 2 with the MVC ring, though it was highly variable (T_{max} 48 [374], C_{max} 22×10^6 [33×10^6] pg/mL), followed by a gradual decline over the first two weeks with a slight rise in the final two weeks (Figure 2B). With the DPV/MVC ring, CVF MVC

concentration peaked consistently on day 1 (T_{max} 26 [28]), with more pronounced C_{max} (97 x 10^6 [124 × 10^6] pg/mL), followed by a gradual decline over the remaining 28 days (Figure 2B). The C_{28D} concentrations for CVF were similar with the two products. The MVC CVF AUC_{inf} (p=0.01) and C_{max} (p<0.001) were greater with the DPV/MVC ring compared to MVC only ring. Three days after ring removal, the CVF MVC concentration was detectable in 3 of 5 subjects in each ring arm having fallen by nearly 2 log_{10} (median 2% of day 28 values) and was below LLOQ thereafter. MVC was detectable in cervical tissue in only 4 of 12 MVC users (range 0.13 to 4.39 ng/mg) and undetectable in DPV/MVC users.

Analysis of cervical tissue in the *ex vivo* challenge assay showed a concentration-response relationship between tissue DPV concentration and viral replication as measured by cumulative p24 corrected for tissue weight on days 7 and 11 of culture from DPV and DPV/MVC ring users (p<0.05, Figure 3, subfigures B and C). There was no significant HIV inhibitory activity in cervical tissue from DPV users on day 4 (Figure 3, subfigure A). Cervical tissue from MVC users showed no drug-associated inhibition of HIV replication over days 4 through 11 of culture (Figure 3, subfigures D-F).

Forty-seven women had vaginal bacterial cultures from at least three visits. The prevalence of pigmented anaerobic Gram negative rods increased significantly during use of the placebo (RR 2.28, CI 1.15–4.49, p=0.02*)* and DPV rings (RR 1.67, CI 1.02-2.73, p=0.04) compared to baseline and day 52. Overall, there was a decrease in prevalence of *E. coli* during ring use (RR 0.50, CI 0.26-0.96, p=0.04). However, no arm was statistically significant. There was no significant change of the Gram stain Nugent score (p=0.27) or *Lactobacillus* over 52 days

(p=0.82) or in the prevalence or quantity of *Candida* species (p=0.72). The amount of biofilm on a subset of 16 rings ranged from scant to confluent but there was no significant difference in the quantity of biofilm, stratified by ring type.

Ninety-four percent of participants were fully adherent by self-report. Mean residual DPV concentrations were 20.6 mg (SD 0.8, n=8) and 21.6 mg (SD 1.6, n=8) in the DPV and DPV/MVC arms, respectively, representing 82% and 86% of the loaded doses. Mean residual MVC concentrations were 95.7 mg (SD 8.0, n=8) and 95.0 mg (SD 7.6) in the MVC and DPV/MVC arms, respectively, representing 96% and 95% of the loaded dose.

DISCUSSION

In this first study of a combination microbicide vaginal ring containing DPV and MVC, vaginal rings containing DPV, MVC, and DPV/MVC were safe and well-tolerated over 28 days with high adherence in healthy, HIV-uninfected women.

While low relative to CVF concentrations, plasma DPV concentrations with the DPV ring rose to 41% of their plateau concentrations within 6 hours following ring insertion, indirectly indicating rapid achievement of tissue and CVF concentrations close to their C_{max}. Assuming the genital tract concentrations achieved are protective, this rapid rise in DPV concentration suggests effectiveness within several hours of ring placement. DPV from both DPV and DPV/MVC rings was detectable in CVF at concentrations at least 100,000-fold above concentrations detectable in plasma, indicating low risk for systemic toxicity.

Median cervical tissue DPV levels at day 28 were approximately 100-fold above the *in vitro* IC_{90}.[30] Tissue-associated DPV inhibited HIV infection in the *ex vivo* challenge assay in a concentration-dependent manner. The lack of HIV inhibitory activity for MVC tissue samples correlates with lack of drug in cervical tissue. Thus, the PD activity ascribed by *ex vivo* challenge assay correlated to tissue concentrations of the two drugs. Establishing the relationship between the *ex vivo* and clinical EC_{50} for DPV will be an important step toward supporting the use of the *ex vivo* challenge assay for predicting clinical activity of candidate microbicides in early clinical trials.

While we could not estimate the half-life of DPV and MVC decay from cervical tissue or CVF following ring removal, the decline was greater than 10-fold by 3 days for all rings, suggesting a restricted window of time between ring removal and sufficient drug levels to inhibit HIV. However, since the method employed for measurement of tissue levels does not differentiate between drug within tissue versus drug adherent to tissue surface, caution is needed in interpreting tissue concentrations and drug activity. Further studies are needed to determine how long DPV or MVC vaginal rings can remain outside the body before drug levels fall below the threshold for protection against HIV acquisition.

The DPV dosage evaluated in this and in the current Phase III trials is based on previous studies showing that 25 mg matrix rings release sufficient quantities of drug predicted to inhibit HIV transmission *in vitro*.[31,32] In selecting the dosage used for a combination DPV/MVC ring, *in vitro* studies showed that the MVC load influences release of DPV due to modified diffusional characteristics of the silicone elastomer network.[33] The MVC dosage in the DPV/MVC ring was

based on matching the *in vitro* release profile of the 25 mg DPV-only ring. The effect of combining two drugs in the matrix ring on the *in vitro* release profile of both drugs was consistent with our *in vivo* findings: DPV/MVC rings had higher peak concentration (C_{max}) and overall drug exposure (AUC_{inf}) in CVF compared to rings with each drug alone, but the concentration at the time of ring removal (C_{28D}) were similar. Compared to the early DPV plateau with the DPV ring, the early peak and gradual fall in concentration in the DPV/MVC ring and MVC ring indicates an inefficient drug delivery profile that results in excess drug exposure early (risking toxicity) and lower concentrations later (risking lack of efficacy).

In addition, MVC is a substrate of p-glycoprotein and has been shown to have much greater permeability in cell cultures in the basolateral to apical direction than in the opposite direction.[34] Since p-glycoprotein is present in the female lower genital tract, there may be active transport of maraviroc out of tissue.[35] This may at least partially explain why MVC was below LLOQ in all cervical tissue biopsies in DPV/MVC ring users, in which case the combination ring will need to be reformulated to release higher levels of MVC to overcome the efflux out of the tissue. Further research is needed to investigate the feasibility of maraviroc as a vaginal microbicide.

The overall safety profile of both DPV and MVC rings is reassuring, as the majority of women had only grade 1 genitourinary AEs with no difference between study arms and there was no difference in grade 2 or higher AEs by study arm. Although there was a decrease in *E. coli* and an increase in anaerobic gram negative rods, these changes were not significant in any of the arms and did not impact the overall change in biofilm development, Nugent score, or the quantity of *Lactobacillus* or *Candida*. This indicates that use of the vaginal rings does not appear

to increase the incidence of bacterial vaginosis or vaginal candidiasis. However, conclusions about safety of MVC-containing rings are limited since MVC concentrations were only consistently detectable in CVF and not in plasma or tissue.

Since this was a PK and safety study, participants were asked to abstain from penetrative intercourse and receptive oral sex throughout the study. Despite these restrictions which limit generalizability, study retention and adherence to ring use were high. It is unclear whether vaginal intercourse may affect PK of drug absorption or release, for example through dilution from semen or displacement of CVF (with drug) with the mechanics of sex. The mean residual drug levels of the DPV ring of 20.6 mg compared to the 25 mg loading dose are encouraging that the DPV ring may contain enough drug for longer periods of use. Additional studies are needed to evaluate drug release beyond 28 days.

Almost all women were adherent with 28 days of ring use by self-report. The high adherence is promising for further development of vaginal rings given the impact of low adherence of daily microbicides on microbicide efficacy.[3] Since adherence to ring use was primarily assessed by self-report, high adherence rates could have been affected by social desirability and response bias. However, residual drug levels in returned rings in this study were comparable to a previous study conducted by IPM that also found approximately 4 mg of dapivirine released from the rings over 28 days,[4] thus the rings were likely used as instructed. In addition, adherence was assessed by both CASI and face-to-face interview, and responses by CASI are expected to be less susceptible to social desirability bias. However, since CVF and cervical tissue DPV levels

rapidly decreased after ring removal, continuous use of the ring will likely remain important for efficacy.

In summary, DPV, but not MVC, delivered by a vaginal ring for 28 days provided dose-dependent inhibition of HIV infection *ex vivo* in cervical tissue. Vaginal rings were safe and well-tolerated across all treatment arms. These data suggest that delivery of NNRTIs via vaginal rings is a promising approach for HIV prevention. More work is needed to determine the appropriate scaling of *ex vivo* and clinical concentration targets (IC_{90}), and to improve the release characteristics of ARVs from the combination and MVC ring.

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FIGURE LEGENDS

Figure 1. Flowchart of participants

* Participant withdrew after Day 29 visit; data prior to withdrawal were included in the analyses.

DPV = dapivirine, MVC = maraviroc

Figure 2. Dapivirine and maraviroc cervicovaginal fluid concentrations over time.

Concentrations below the limits of quantification are not shown.

CVF, cervicovaginal fluid; DPV, dapivirine; MVC, maraviroc

A. Cervicovaginal fluid dapivirine concentration vs. time plot (mean + SD)
B. Cervicovaginal fluid maraviroc concentration vs. time plot (mean + SD)

Figure 3. Pharmacodynamic and pharmacokinetic correlations from fresh cervical tissue collected from women using DPV/MVC (open triangle), DPV only (black circle) and MVC only (grey square) vaginal rings. *Ex vivo* p24 and drug concentrations were measured from tissue samples taken on Day 28 following the active treatment. Supernatant p24 was collected on Days 4, 7, and 11 of the *ex vivo* HIV challenge assay. DPV (A-C, Days 4, 7, and 11) and MVC (D-F, Days 4, 7, and 11) cervical tissue concentrations (Log_{10} ng/mL) are plotted against p24 log_{10} pg/mL. The pharmacokinetic (MVC or DPV) and pharmacodynamic (p24) data were fit with an inverse, linear least squares regression at each day of the *ex vivo* assay with significance noted. Data below the limit of quantification were imputed as half the lower limit of quantification (DPV = 1.6 log_{10} ng/mL; MVC = 1.0 log_{10} ng/mL) and indicated with a vertical dotted line in the figures.

DPV, dapivirine; MVC, maraviroc; LLOQ, lower limit of quantification; VR, vaginal ring

Table 1. Demographics of study participants in MTN-013/IPM 026 by study arm

	Placebo	Dapivirine	Maraviroc	Dapivirine + Maraviroc	All arms
Participants enrolled	12	12	12	12	48
Mean age in years (SD)	28.7 (4.4)	33.6 (5.7)	28.4 (6.7)	27.7 (6.7)	29.6 (6.2)
18-24 years (n, %)	2 (17%)	1 (8%)	4 (33%)	6 (50%)	13 (27%)
25-29 years (n, %)	5 (42%)	2 (17%)	4 (33%)	3 (25%)	14 (29%)
30-34 years (n, %)	4 (33%)	3 (25%)	1 (8%)	0 (0%)	8 (17%)
35-40 years (n, %)	1 (8%)	6 (50%)	3 (25%)	3 (25%)	13 (27%)
Race					
White (n, %)	5 (42%)	6 (50%)	6 (50%)	7 (58%)	24 (50%)
Black or African American (n, %)	6 (50%)	5 (42%)	5 (42%)	2 (17%)	18 (38%)
Asian (n, %)	0 (0%)	0 (0%)	0 (0%)	2 (17%)	2 (4%)
Other (n, %)	1 (8%)	1 (8%)	1 (8%)	1 (8%)	4 (8%)

Table 2. Summary of pharmacokinetic parameters at sampled anatomic sites [Median (interquartile range)]

Drug	Specimen	Ring type	N	AUC_{inf} hr-pg/mL	p-value AUC_{inf}	T_{max} hr	p-value T_{max}	C_{max} pg/mL	p-value C_{max}	C_{28D} pg/mL	p-value C_{28D}
DPV	Plasma	DPV	12	13.3E4 (2.9E4)	0.01	421 (464)	<0.001	231 (46)	0.08	175 (45)	0.005
		DPV/MVC	12	8.6E4 (2.7E4)		35 (41)		294 (144)		88 (47)	
	CVF	DPV	12	4.4E9 (9.2E9)	0.08	76 (582)	0.05	24E6 (22E6)	0.002	5.7E6 (18.7E6)	0.67
		DPV/MVC	12	12.3E9 (11.9E9)		37 (49)		77E6 (76E6)		6.8E6 (17.0E6)	
	CT	DPV	12	-		-		-		0.6E6 (0.9E6)	0.98
		DPV/MVC	12	-		-		-		0.5E6 (1.1E6)	
MVC	Plasma	MVC	12	BLQ		-		BLQ		BLQ	
		DPV/MVC	12	BLQ		-		BLQ		BLQ	
	CVF	MVC	12	3.7E9 (3.4E9)	0.01	48 (374)	0.14	22E6 (33E6)	<0.001	2.5E6 (4.0E6)	0.29
		DPV/MVC	12	6.2E9 (5.6E9)		26 (28)		97E6 (124E6)		1.1E6 (1.0E6)	
	CT	MVC	12	-		-		-		BLQ	
		DPV/MVC	12	-		-		-		BLQ	

DPV, dapivirine; MVC, maraviroc; CVF, cervicovaginal fluid; CT, cervical tissue; BLQ, below limits of quantification

Listed p-values reflect the comparison between the single drug (DPV or MVC) ring and the combination drug (DPV/MVC) ring using Mann-Whitney U tests with exact significance.

E4 indicates x 10^4; E6 indicates x 10^6; E9 indicates x 10^9

The lower limits of quantification for DPV in plasma and CVF were 20 pg/mL and 0.05 ng/tear strip (2 pg/mg based on typical sample weights), respectively. The lower limits of quantification for MVC in plasma and CVF were 0.5 ng/mL and 0.025 ng/tear strip (1 pg/mg), respectively.