Clinical and Virologic Outcomes Following Initiation of Antiretroviral Therapy Among Seroconverters in the Microbicide Trials Network-020 Phase III Trial of the Dapivirine Vaginal Ring

Sharon A. Riddler,¹ Jennifer E. Balkus,² Urvi M. Parikh,¹ John W. Mellors,¹ Carolyn Akello,³ Sofia Dadabhai,⁴ Felix Mhlana,⁵ Gita Ramjee,⁶ Ashley J. Mayo,⁷ Edward Livant,⁸ Amy L. Heaps,¹ Colin O'Rourke,^{2,a} and Jared M. Baeten², for the MTN-015 and MTN-020/ASPIRE Study Teams

¹University of Pittsburgh, Pennsylvania; ²University of Washington, Seattle; ³Makerere University–Johns Hopkins University Research Collaboration, Kampala, Uganda; ⁴Johns Hopkins Bloomberg School of Public Health, Blantyre, Malawi; ⁵University of Zimbabwe, Harare; ⁶South African Medical Research Council, Durban; ⁷FHI 360, Durham, North Carolina; and ⁸Magee-Womens Research Institute & Foundation, Pittsburgh, Pennsylvania

Background. A vaginal ring containing dapivirine, a non-nucleoside human immunodeficiency virus (HIV)-1 reverse transcriptase inhibitor (NNRTI), was safe and effective in preventing HIV-1 infection in African women. We examined the impact of dapivirine ring use at the time of HIV-1 acquisition on subsequent HIV-1 disease progression and responses to NNRTI-containing antiretroviral therapy (ART).

Methods. HIV-1 disease progression and virologic failure following initiation of ART were assessed among women who acquired HIV-1 while participating in Microbicide Trials Network-020, a randomized, placebo-controlled trial of a monthly, dapivirine vaginal ring.

Results. Among the 158 participants who acquired HIV-1 (65 dapivirine, 93 placebo), no differences between dapivirine and placebo participants were observed in CD4+ cell counts or plasma HIV-1 RNA over the first year after infection (prior to ART). During follow-up, 100/158 (63%) participants initiated NNRTI-containing ART (dapivirine: 39/65; placebo: 61/93); the median time to HIV-1 RNA <200 copies/ml was approximately 90 days for both dapivirine and placebo ring recipients (log-rank $P = .40$). Among the 81 participants with at least 6 months of post-ART follow-up, 19 (24%) experienced virologic failure (dapivirine: 6/32, 19%; placebo: 13/39, 27%; $P = .42$).

Conclusions. The acquisition of HIV-1 infection during dapivirine or placebo treatment in ASPIRE did not lead to differences in HIV-1 disease progression. After the initiation of NNRTI-containing ART, dapivirine and placebo participants had similar times to virologic suppression and risks of virologic failure. These results provide reassurance that NNRTI-based ART regimens are effective among women who acquired HIV-1 while receiving the dapivirine vaginal ring.

Clinical Trials Registration. NCT016170096 and NCT00514098.

Keywords. antiretroviral therapy outcomes; antiretroviral treatment; dapivirine vaginal ring; HIV drug resistance.

The development of antiretroviral medications for the prevention of human immunodeficiency virus (HIV)-1 infection has great potential for reducing the HIV-1 incidence in high-risk populations. Taken orally, tenofovir disoproxil fumarate in combination with emtricitabine (TDF/FTC) has demonstrated efficacy in multiple studies and has been approved and widely recommended for individuals at increased risk of HIV-1 acquisition [1–5]. More recently, a vaginal ring containing the non-nucleoside reverse transcriptase inhibitor (NNRTI) dapivirine

was found to be effective for the prevention of HIV-1 infection in 2 phase III, randomized, placebo-controlled clinical trials—Microbicide Trials Network (MTN)-020/ A Study to Prevent Infection with a Ring for Extended Use (ASPIRE) and International Partnership for Microbicides (IPM) 027/The Ring Study—with overall reductions in HIV infections of 27% and 31%, respectively [6, 7].

In addition to efficacy and safety, antiretroviral drugs investigated for prevention must also be evaluated for their impact on early and long-term outcomes among those who become HIV-1 infected while using antiretroviral-based prevention methods. In particular, the potential for HIV-1 resistance to antiretroviral medications and the impact of such resistance on future treatments for HIV-1 infections have been persistent concerns [8, 9]. Efficacy trials of TDF/FTC and dapivirine have reported few instances of drug-resistance mutations at the time of seroconversion among trial participants who were HIV-1 negative at the time of randomization to the active study products [1–3]. In the

Received 7 July 2018; editorial decision 11 October 2018; accepted 18 October 2018; published online October 22, 2018.

Presented in part: Conference on Retroviruses and Opportunistic Infections, Seattle, WA, 13–16 February 2017. Abstract 952.

^aPresent affiliation: Benaroya Research Institute, Seattle, Washington.

Correspondence: S. A. Riddler, 3520 Fifth Ave, Suite 510, Pittsburgh, PA 15213 (riddler@pitt.edu).

Clinical Infectious Diseases® 2019;69(3):523–9

© The Author(s) 2018. Published by Oxford University Press for the Infectious Diseases Society of America. All rights reserved. For permissions, e-mail: journals.permissions@oup.com. DOI: 10.1093/cid/ciy909

ASPIRE and Ring studies, the rates of NNRTI mutations at the time of seroconversion were similar among dapivirine ring and placebo participants. Longitudinal observations of participants who acquired HIV-1 while receiving antiretroviral-based prevention provide the opportunity to determine the impact of these effective prevention interventions on HIV-1 disease progression and subsequent responses to antiretroviral treatment (ART).

NNRTIs used for prevention require considerable attention to longitudinal outcomes, due to the lower genetic barrier to resistance, as compared to other classes of antiretrovirals. Single-dose nevirapine (sdNVP), used for the prevention of mother-to-child transmission of HIV-1, was associated with an approximately 3-fold increase in the risk of virologic failure to first-line, NNRTI-containing ART when ART was initiated by women within 6–12 months of receiving sdNVP [10]. The infants of HIV-1-infected women who acquired HIV-1 despite sdNVP regimens were also more likely to have virologic failure by 6 months after initiation of NVP-based ART [10, 11].

NNRTI-containing regimens continue to be first-line ARTs in many regions of the world, adding to the need to assess outcomes in women with dapivirine exposure who seroconvert and subsequently initiate ART. We assessed HIV-1 disease progression, the occurrence of virologic failure, and HIV-1 drug resistance following the initiation of ART among women who acquired HIV-1 during participation in the ASPIRE trial of the dapivirine vaginal ring.

METHODS

Study Design and Participants

The ASPIRE trial enrolled 2629 healthy, HIV-1-seronegative women at 15 sites in 4 African countries (ClinicalTrials.gov number, NCT016170096) [6, 12]. Participants were randomized to receive a monthly vaginal ring containing either 25 mg dapivirine or a matching placebo. As part of the efforts to monitor the overall use of the study product during the trial, plasma dapivirine concentrations were assessed every 3 months [6, 13]. Participants underwent monthly HIV-1 antibody testing, and the study product was withheld at the time of the first positive rapid HIV-1 antibody test and was permanently discontinued when a HIV-1 infection was confirmed.

Women who were HIV-1 infected at enrollment into ASPIRE or those whose infection was detected at the final visit in ASPIRE after the study product was no longer used were not included in the current analyses. ASPIRE participants with HIV-1 infections were offered enrollment into an ongoing, observational cohort study for extended follow-up (MTN-015; ClinicalTrials.gov number NCT00514098) [14]. Participants who joined MTN-015 had study visits at 1, 3, and 6 months and at every 6 months after the first positive rapid-HIV-1-antibody test, with additional visits at the time of ART initiation. ART initiation and management were based on local and/or World Health Organization

guidelines [15]. Participants who declined participation in MTN-015 were followed in ASPIRE until their originally-scheduled study exit date, and underwent blood testing for HIV-1 RNA and CD4 cell counts at 1, 3, 6, 12, and 18 months after the first positive rapid-HIV-1-antibody test was documented.

Human Immunodeficiency Virus-1 Genotype Analysis for Drug Resistance

Standard resistance testing using a laboratory-developed Sanger-sequencing-based population genotype assay (*protease* amino acids 1–99 and *RT* amino acids 1–333) optimized for non-B HIV-1 subtypes was performed on plasma samples collected at the time of confirmation of seroconversion in ASPIRE and at the time of virologic failure in MTN-015 [6]. When available, an additional specimen, obtained 2 or more months after seroconversion from participants with NNRTI-associated resistance mutations at seroconversion, was tested. Drug-resistance mutations in the HIV-1 Group M subtype were identified using the Stanford HIV-1 Drug Resistance Database, version 8.4 [16].

Statistical Analysis

For the analysis, the estimated seroconversion date was defined as the midpoint between the last negative and first positive rapid-HIV-1 test in ASPIRE. Descriptive statistics were used to summarize key factors at baseline and during follow-up. Categorical variables were compared by ASPIRE study arm using a chi-squared test or Fisher's exact test, as appropriate, and time-to-event outcomes were compared using the log-rank test. We assessed HIV-1 disease progression by ASPIRE study arm from the estimated date of seroconversion through the 12-month follow-up study visit, including data collected up to 15 months following the estimated date of seroconversion, since the follow-up visit schedule was based on the date of the first positive rapid-HIV-1 test rather than estimated seroconversion. Changes in CD4+ T cell count per mm³ and plasma HIV-1 RNA (log₁₀ copies/ml) by time and ASPIRE study arm were assessed using generalized, linear, mixed-effects models using a gamma family for CD4+ T cell counts and Gaussian family for log₁₀ copies/ml plasma HIV-1 RNA. Correlations caused by repeated measurements were accounted for by including a random intercept term for each individual participant. Only measurements taken between 3 months and up to 15 months from the estimated date of seroconversion were included in the models, and time was assumed to act linearly on the log scale. The interaction between time and ASPIRE study arm was included, allowing for potentially separate trajectories by arm. Follow-up was censored at the date ART was initiated or at loss to follow-up.

Kaplan-Meier curves and unadjusted Cox proportional hazard models, stratified by country, were generated to assess the time from the estimated HIV-1 seroconversion to the following outcomes: first occurrence of CD4+ cell count ≤350 cell/mm³, the initiation of ART for any reason, and a composite outcome, defined as the first of these 2 events. For models assessing

the first occurrence of CD4+ cell count ≤ 350 cell/mm³, participant follow-up was censored at ART initiation.

Among women who initiated ART and who had at least 6 months of follow-up on ART, descriptive statistics and a Fisher's exact test were used to assess the proportion of participants with virologic failure by ASPIRE study arm. Virologic failure was defined as either a lack of suppression of plasma HIV-1 RNA, to <200 copies/ml after 6 months of ART, or a plasma HIV-1 RNA rebound, to ≥ 200 copies/ml at any time after suppression (rebound).

The above analyses were repeated as part of a sensitivity analysis that compared participant outcomes separately, by plasma dapivirine concentrations, measured at quarterly visits in ASPIRE using the results from the most proximate visit on or before the date of estimated seroconversion. Groupings were formed based on dapivirine plasma concentration values, where a concentration of >95 pg/per ml at or before the estimated seroconversion date was considered to be an indicator of dapivirine ring use and a concentration of 95 pg/ ml or less was considered to be an indicator of non-use [6].

RESULTS

Study Population

Of 168 participants with incident HIV-1 infections in ASPIRE, 158 had at least 1 post-seroconversion assessment and were included in these analyses (Figure 1). Participant characteristics are provided in Table 1. The mean age was 23 years and the majority of participants were enrolled in South Africa (76%). The overall median follow-up time after seroconversion was 34.4 months. As expected, the follow-up time was longer in those enrolled in MTN-015 than for those not enrolled (median follow-up time: enrolled into MTN-015 [n = 121]: 38.6 months; followed only in ASPIRE [n = 37]: 8.0 months). The majority of participants initiated ART (n = 100, 63%); of these, 81 (81%) had 6 months or more of follow-up after ART initiation.

Figure 1. Disposition of study participants with incident HIV-1 infection during MTN-020/ASPIRE. Abbreviations: ART, antiretroviral therapy; ASPIRE, A Study to Prevent Infection with a Ring for Extended Use; HIV, human immunodeficiency virus; MTN, Microbicide Trials Network; VL, viral load.

Table 1. Characteristics of the Analysis Study Population

	All (N = 158)	Placebo (n = 93)	Dapivirine (n = 65)
Age, in years	23 (21, 27)	25 (22, 27)	22 (20, 27)
Clade C virus, n (%)	145/155 (94%)	86/92 (93%)	59/63 (94%)
Initial HIV RNA, as log ₁₀ copies/ml	4.6 (3.9, 5.2)	4.6 (4.0, 5.2)	4.6 (3.6, 5.1)
Initial CD4 count, as cells/mm ³ (n = 157)	547 (429, 707)	523 (396, 674)	601 (464, 793)
Median follow up after seroconversion, in months	34.4 (18.1, 43.6)	35.6 (24.4, 44.5)	31.5 (17.6, 43.4)
Initiated ART, n (%)	100 (63%)	61 (66%)	39 (60%)
Greater than 6 months ART ^a	81 (81%)	49 (80%)	32 (82%)
Virologic failure ^a	19/81 (24%)	13/49 (27%)	6/32 (19%)

Values are provided as median (interquartile range), unless otherwise noted.
Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus.
^aProportion among those initiating ART.

Human Immunodeficiency Virus–1 Disease Progression

The analysis of CD4+ T cell and HIV-1 RNA trajectories were based on 144 participants who had at least 12 months of follow-up (81 placebo and 63 dapivirine ring). Participant data were censored at the time of initiation of ART. Models comparing CD4+ T cell counts and plasma HIV-1 RNA showed no differences between placebo and dapivirine recipients for the initial CD4+ cell count ($P = .46$) and HIV-1 RNA ($P = .79$), or the trajectory of the CD4+ cell count ($P = .67$) and HIV-1 RNA ($P = .50$), through up to 15 months following the date of estimated seroconversion (Figure 2). Additionally, there were no differences between the ASPIRE treatment arms in the cumulative incidence of or the median time to CD4+ T cells ≤ 350 or ART initiation, or for the composite endpoint of CD4+ T cells ≤ 350 or ART initiation (data not shown). Clinical, HIV-related or acquired immunodeficiency syndrome events were uncommon. Among 121 women enrolled into the MTN-015 cohort, 12 participants experienced a World Health Organization Stage 3 or 4 condition: 8 in the placebo group and 4 in the dapivirine group. These included unexplained, severe weight loss ($>10\%$ body weight); unexplained, moderate weight loss ($<10\%$ body weight); papular pruritic eruption; and pulmonary tuberculosis.

Persistence of Non-nucleoside Reverse Transcriptase Inhibitor Mutations Detected at Seroconversion

In the ASPIRE trial, 18 (11%) participants with incident HIV-1 infections had 1 or more NNRTI resistance mutations detected in plasma at the time of seroconversion, with no difference between the dapivirine and placebo ring arms [6]. Additional genotypic resistance testing was performed on plasma samples from 17 of 18 participants, collected between 9 to 108 weeks after seroconversion (median 47 weeks; Table 2). The NNRTI-associated mutations present at the time of seroconversion were again identified at the follow-up testing time point for all 17 participants. There

Figure 2. Individual participant trajectories of (A) CD4+ T cell count and (B) plasma HIV-1 RNA ($\log_{10}$ copies/mL) from 3 to 15 months following estimated seroconversion, and estimated trends based on fixed-effects for measurements 3 or more months from estimated seroconversion. Abbreviation: HIV, human immunodeficiency virus.

was 1 participant, randomized to the dapivirine ring, who had an isolated H221Y mutation at seroconversion and subsequently developed NNRTI-associated mutations (V106M

and Y181C), which were detected at virologic failure 6 weeks after initiating ART with efavirenz, FTC, and TDF, as described below.

Table 2. Follow-up Genotypic Resistance Testing Among Participants With NNRTI Mutations at Seroconversion

NNRTI Mutations at Seroconversion	NNRTI Mutations at Follow-up	ASPIRE Arm	Weeks Between Seroconversion and Follow-up Samples	Initiated ART Prior to Follow-up Sample
V108IV, E138A	E138A	Dapivirine	108	Yes
K103S, V106M	K103S, V106M	Dapivirine	76	No
H221Y	V106M, Y181Y/C, H221Y	Dapivirine	50	Yes
K101E, E138G	K101E, E138G	Dapivirine	26	Yes
E138A, V179D	E138A, V179D	Dapivirine	26	No
V90I, K103N	V90I, K103N	Dapivirine	14	No ^c
E138A, V179IT	E138A, V179I/T	Dapivirine	9	No
V90I, K103N	NA ^a	Dapivirine	...	...
E138A	E138A	Placebo	98	Yes
V179D	V179D	Placebo	95	Yes
K101E, E138A	K101E, E138A	Placebo	80	No ^c
E138A	E138A ^b	Placebo	52	No
E138A	E138A	Placebo	52	No
V90I	V90I	Placebo	47	NA
E138A	E138A	Placebo	39	No
K103N	K103N	Placebo	37	Yes
H221Y	H221Y	Placebo	24	Yes
V179D	V179D	Placebo	17	No ^c

Abbreviations: ART, antiretroviral therapy; ASPIRE, A Study to Prevent Infection with a Ring for Extended Use; HIV, human immunodeficiency virus; NA, not available; NNRTI, non-nucleoside reverse transcriptase inhibitor.

^aThis participant had HIV-1 RNA <200 copies/mL at all time points after seroconversion and follow-up genotype was not done.

^bAdditional mutation in HIV-1 protease Q58E.

^cThese participants initiated ART after the follow-up sample was obtained.

Table 3. Resistance Mutations Detected in Plasma Collected at Seroconversion and Virologic Failure

ASPIRE Arm	Initial ART Regimen	Mutations at Seroconversion	Mutations at Virologic Failure
Dapivirine	EFV/FTC/TDF	V108I/V, E138A	E138A
Dapivirine	EFV/FTC/TDF	H221Y	V106M, Y181Y/C, H221Y
Dapivirine	EFV/FTC/TDF	None	None
Dapivirine	EFV/FTC/TDF	None	K103N
Dapivirine	EFV/FTC/TDF	None	None
Placebo	EFV/LAM/TDF	None	K103K/N
Placebo	EFV/FTC/TDF	None	None
Placebo	NVP/LAM/d4T	None	G190G/A
Placebo	NVP/LAM/d4T	None	K103K/N
Placebo	EFV/LAM/TDF	None	K103N
Placebo	EFV/FTC/TDF	None	None
Placebo	EFV/FTC/TDF	None	None
Placebo	EFV/FTC/TDF	None	Y188Y/H
Placebo	EFV/FTC/TDF	None	V106V/M, Y188Y/F/H/L
Placebo	EFV/LAM/TDF	None	None
Placebo	EFV/FTC/TDF	None	None

There were 19 participants that had virologic failure; 2 samples that were not available; and 1 sample that failed to amplify.

Abbreviations: ART, antiretroviral therapy; ASPIRE, A Study to Prevent Infection with a Ring for Extended Use; d4T, stavudine; EFV, efavirenz; FTC, emtricitabine; HIV, human immunodeficiency virus; LAM, lamivudine; NVP, nevirapine; TDF, tenofovir disoproxil fumarate.

Antiretroviral Therapy Response and Virologic Failure

All participants who initiated ART received 1 NNRTI (efavirenz in 93% and nevirapine in 7%) and 2 nucleoside reverse transcriptase inhibitors (NRTIs). The NRTIs were TDF and FTC or lamivudine for all participants except for 2, who received stavudine and lamivudine instead. Overall, the median follow-up time on ART was 18.8 months. Among the 81 participants with 6 or more months of observation time on ART (32 in the dapivirine group and 49 in the placebo group), the median time from ART initiation to viral suppression was 90 days (95% confidence interval 65–102) in the placebo group and 90 days (95% confidence interval 29–104) in the dapivirine group (log-rank $P = .40$). The overall rate of virologic failure was 24% (19 of 81), with no significant difference between ASPIRE treatment arms (dapivirine: 6/32, 19%; placebo: 13/49, 27%; chi-square $P = .42$). The virologic failure events included 6 participants who did not achieve plasma HIV-1 RNA <200 copies/ml after 6 months on ART and 13 participants who had viral rebounds after initial viral suppression. Of 18 (56%) participants with virus encoding NNRTI–drug-resistance mutations at seroconversion, 10

initiated ART (4 in the dapivirine group and 6 in the placebo group). Of these 10 participants (30%; 2 in the dapivirine group and 1 in the placebo group), 3 experienced virologic failure compared with 16 of 71 (22.3%) participants with wild-type virus at seroconversion (Fisher's exact $P = .69$).

Resistance genotype results were available for 16 of 19 participants with virologic failure after ART initiation (Table 3). Of these 16 participants, 9 had 1 or more NNRTI–drug-resistance mutations, with 8 of these encoding mutations that were treatment-emergent (not present in pre-ART samples collected near the time of seroconversion). As noted above, however, 1 of the 9 had an isolated H221Y mutation at seroconversion, followed by V106M and Y181C mutations at virologic failure. Whether the H221Y mutation contributed to virologic failure is unclear. Overall, the most common treatment-emergent NNRTI mutation was K103N, which was present in 4 participants.

Plasma Dapivirine Concentration at Seroconversion

Among the 65 dapivirine ring participants with seroconversion, 16 (25%) had missing information for the dapivirine plasma concentration on ASPIRE; of the remaining 49 participants with available measurements, 36 (74%) had dapivirine concentrations >95 pg/ml and 13 (26%) had concentrations between 0–95 pg/ml. The occurrence of NNRTI-resistance mutations at seroconversion was similar between the dapivirine concentration groups (Table 4) with 5 of 36 (13.9%) in the >95 pg/ml group having an NNRTI resistance mutation, compared with 10 of 93 (10.8%) in the placebo group. Virologic failure on ART was similarly distributed across the dapivirine concentration groups, with the lowest proportion among participants with dapivirine concentrations >95 pg/ml; however, the number of individuals in each group was small (Table 4).

DISCUSSION

Although the dapivirine vaginal ring has shown efficacy for the prevention of HIV-1 infection among women in sub-Saharan Africa, it is important to investigate potential effects of the ring on drug resistance and subsequent responses to ART among women who are not protected [6, 7]. In the current study's analyses of the longitudinal outcomes of individuals who became infected with HIV-1 during participation in the ASPIRE trial, the dapivirine ring was not associated with significant differences, as compared to placebo recipients, in HIV-1 disease parameters, including CD4+ cell count and plasma HIV-1 RNA during the first year after

Table 4. Summaries of the Occurrence of Non-nucleoside Reverse Transcriptase Inhibitor Resistance Mutations at the Time of Seroconversion and Virologic Failure by Dapivirine Concentration

	Overall	Placebo	Plasma Dapivirine Concentration (pg/ml)		
			Missing	0–95	>95
Resistance mutation at seroconversion	18/158 (11.4%)	10/93 (10.8%)	2/16 (12.5%)	1/13 (7.7%)	5/36 (13.9%)
Virologic failure	19/81 (23.5%)	13/49 (26.5%)	3/11 (27.3%)	1/4 (25.0%)	2/17 (11.8%)

seroconversion, prior to the initiation of ART. Importantly, the virologic outcomes following the initiation of NNRTI-containing ART, including time to virologic suppression and the rate of virologic failure, were not different among participants who received the dapivirine ring and those who received the placebo.

Several studies have reported HIV-1 disease progression outcomes for individuals who acquire HIV-1 infection during randomized, clinical trials of tenofovir-based pre-exposure prophylaxis (PrEP) [17–19]. In general, no significant impact of PrEP on the clinical course of HIV-1 has been observed; however, no prior study has included longitudinal data after ART initiation. Recent updates to the World Health Organization and South African ART treatment guidelines have resulted in shorter times to ART initiation, as evidenced in the current study by more than 60% of the participants infected during the ASPIRE study initiating ART within 3 years of seroconversion, compared to only approximately 25% from the MTN-003 trial, conducted several years earlier [19].

Prior to the development of the dapivirine vaginal ring, nearly all of the clinical experience with NNRTI drugs for HIV-1 prevention was in the use of oral nevirapine in the perinatal period to prevent HIV-1 transmission from an HIV-1-infected mother to her infant. There are important differences between the use of sdNVP for the prevention of maternal-to-child transmission of HIV-1 and the topical use of the dapivirine vaginal ring for the prevention of HIV-1 in uninfected women. The plasma levels of nevirapine in women in the perinatal period and in infants during the perinatal and early post-natal periods are high, with a prolonged period of drug exposure resulting in frequent selection of a resistant virus [20–24]. By contrast, the systemic exposure to dapivirine with intravaginal ring use is low, with plasma levels well below the concentrations required to exert an antiviral effect, thus limiting the selection of a resistant virus [25].

A potential reason for the lack of impact of the dapivirine ring on virologic outcomes before and after ART initiation is nonadherence to the ring, resulting in minimal exposure to dapivirine. To assess this possibility, we stratified analyses by plasma dapivirine levels. The majority (74%) of participants in the current study that were randomized to receive the dapivirine ring had plasma dapivirine levels >95 pg/mL, indicating recent dapivirine ring use, and we did not observe differences in the rates of resistance mutation occurrences or virologic failures among women with high plasma dapivirine concentrations, as compared to placebo.

The frequency of NNRTI mutations in viruses from seroconverters from ASPIRE did not differ by arm, indicating that the NNRTI resistance was likely transmitted, and not selected by dapivirine ring use [6]. The polymorphism E138A was the most common mutation observed in samples collected at the time of seroconversion; however, its frequency also did not differ by ASPIRE study arm. Although the NNRTI mutations were maintained over time, participants harboring

viruses with mutations were not more likely to experience virologic failure while receiving ART than participants infected with wild-type viruses. The mutations observed at seroconversion and virologic failure are consistent with circulating NNRTI mutations and NNRTI mutations that are common in first-line ART failures [26]. As oral PrEP and, potentially, the dapivirine vaginal ring transition to widespread use outside of controlled trial settings, it will be important to continue to monitor both the frequency of resistance-associated mutations in those experiencing seroconversion and subsequent ART failures.

Our study has several important limitations. The data were observational, and not all eligible participants enrolled into MTN-015, resulting in shorter follow-up times for those who did not participate. We used quarterly plasma dapivirine levels, obtained in APSIRE, to assess adherence; however, a substantial minority of the participants had missing data and, even for those with data, the pattern of ring use around the time of seroconversion could not be precisely determined. Although these analyses include more seroconverters than prior studies, the overall number of participants with NNRTI mutations at the time of seroconversion and the number who experienced virologic failure on ART were both relatively small. To date, the genotypic resistance testing has been limited to standard assays in plasma; additional testing using sensitive assays for minority variants and assessing for the presence of drug-resistance mutations from mucosal samples may be informative.

NNRTI-based antiretroviral therapy is frequently used for first-line ART in many regions of the world. Our results provide reassurance that NNRTI regimens are effective in the setting of HIV-1 infections acquired during prophylactic treatment with a dapivirine vaginal ring; nonetheless, continued diligence is warranted in monitoring both antiretroviral prevention failure and, later, ART failure for drug resistance during roll-out of oral PrEP and the dapivirine vaginal ring.

Notes

The study team leadership for A Study to Prevent Infection with a Ring for Extended Use (ASPIRE) consists of Jared Baeten, University of Washington (Protocol Chair); Thesla Palanee-Phillips, Wits Reproductive Health and HIV Institute (Protocol Co-chair); Elizabeth Brown, Fred Hutchinson Cancer Research Center (Protocol Statistician); Lydia Soto-Torres, US National Institute of Allergy and Infectious Diseases (Medical Officer); and Katie Schwartz, FHI 360 (Clinical Research Manager). The Microbicide Trials Network (MTN)-015 study team leadership consists of Sharon Riddler, University of Pittsburgh (Protocol Chair); Jennifer Balkus, Fred Hutchinson Cancer Research Center (Protocol Statistician); Jeanna Piper, US National Institute of Allergy and Infectious Diseases (Medical Officer); and Ashley Mayo, FHI 360 (Clinical Research Manager).

The study sites and site investigators of record are Bonus Makanani, from the Malawi, Blantyre site (Johns Hopkins University, Queen Elizabeth Hospital); Francis Martinson and Lameck Chinula, from the Malawi, Lilongwe site (University of North Carolina, Chapel Hill); Linda-Gail Bekker, from the South Africa, Cape Town site (University of Cape Town); Vaneshree Govender, Samantha Siva, Zakir Gaffoor, Logashvari Naidoo, Arendevi Pather, Bomkazi Tutshana, Jayajothi Moodley, Kubashini Woeber, and Nitesha Jeenarain, from the South Africa, Durban–Botha's Hill, Chatsworth, Isipingo, Tongaat, Umkomaas, and Verulam sites (South African Medical Research Council); Gonasagrie Nair

and Leila Mansoor, from the South Africa, Durban, eThekweni site (Center for the AIDS Programme for Research in South Africa); Thesla Palanee-Phillips, from the South Africa, Johannesburg site (Wits Reproductive Health and HIV Institute, University of the Witwatersrand); Flavia Matovu Kiweewa and Clemensia Nakabiito, from the Uganda, Kampala site (John Hopkins University, Makerere University); Nyaradzo Mgodini, from the Zimbabwe, Chitungwiza, Seke South, and Zengeza sites (University of Zimbabwe College of Health Sciences Clinical Trials Research Centre); and Felix Mhlanga, from the Zimbabwe, Harare, Spilhaus site (University of Zimbabwe College of Health Sciences Clinical Trials Research Centre). Data management was provided by The Statistical Center for HIV/AIDS Research & Prevention (Fred Hutchinson Cancer Research Center, Seattle, Washington) and site laboratory oversight was provided by the Microbicide Trials Network Laboratory Center (Pittsburgh, Pennsylvania).

Acknowledgments. The authors and the Microbicide Trials Network (MTN)-015 and MTN-020 study teams thank the study participants for their dedication to these studies. The authors thank the study staff and investigators from the 15 participating MTN sites and appreciate the support of the ASPIRE co-chairs and team and the MTN leadership. Additionally, the authors recognize the contributions of Marla Husnik, Danny Szydio, Janne Abullarade, Cliff Kelly, and Karen Patterson (Statistical Center for HIV/AIDS Research and Prevention); Lisa Levy (FHI 360); and Vanessa Elharrar and Jeanna Piper (National Institutes of Health, Division of AIDS).

Disclaimer. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Financial support. The study was designed and implemented by the Microbicide Trials Network (MTN) funded by the National Institute of Allergy and Infectious Diseases through individual grants (UM1AI068633, UM1AI068615, and UM1AI106707), with co-funding from the Eunice Kennedy Shriver National Institute of Child Health and Human Development and the National Institute of Mental Health, all components of the US National Institutes of Health. The International Partnership for Microbicides developed the dapivirine vaginal ring and supplied the study rings for A Study to Prevent Infection with a Ring for Extended Use (ASPIRE).

Potential conflicts of interest. S. A. R. has received research grants from Gilead Sciences and GlaxoSmithKline. J. W. M. is a consultant to and has received grant support from Gilead Sciences and owns share options in Co-Crystal Pharma, Inc. J. E. B. has received personal fees from Lupin Pharmaceuticals, outside the submitted work. J. M. B. serves on the advisory board of Gilead Sciences. U. M. P. has received a grant from United States Agency for International Development and payments from the International Partnership for Microbicides, both outside the submitted work. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

- Baeten JM, Donnell D, Ndase P, et al; Partners PrEP Study Team. Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. *N Engl J Med* **2012**; 367:399–410.
- Grant RM, Lama JR, Anderson PL, et al; iPrEx Study Team. Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. *N Engl J Med* **2010**; 363:2587–99.
- Thigpen MC, Kebaabetswe PM, Paxton LA, et al; TDF2 Study Group. Antiretroviral preexposure prophylaxis for heterosexual HIV transmission in Botswana. *N Engl J Med* **2012**; 367:423–34.
- World Health Organization. Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV. **2015**.
- Marrazzo JM, del Rio C, Holtgrave DR, et al; International Antiviral Society-USA Panel. HIV prevention in clinical care settings: 2014 recommendations of the International Antiviral Society-USA Panel. *JAMA* **2014**; 312:390–409.
- Baeten JM, Palanee-Phillips T, Brown ER, et al; MTN-020/ASPIRE Study Team. Use of a vaginal ring containing dapivirine for HIV-1 prevention in women. *N Engl J Med* **2016**; 375:2121–32.
- Nel A, Bekker LG, Bukusi E, et al. Safety, acceptability and adherence of dapivirine vaginal ring in a microbicide clinical trial conducted in multiple countries in Sub-Saharan Africa. *PLoS One* **2016**; 11:e0147743.
- Grant RM, Liegler T. Weighing the risk of drug resistance with the benefits of HIV preexposure prophylaxis. *J Infect Dis* **2015**; 211:1202–4.
- Parikh UM, Mellors JW. Should we fear resistance from tenofovir/emtricitabine preexposure prophylaxis? *Curr Opin HIV AIDS* **2016**; 11:49–55.
- Lockman S, Shapiro RL, Smeaton LM, et al. Response to antiretroviral therapy after a single, peripartum dose of nevirapine. *N Engl J Med* **2007**; 356:135–47.
- Palumbo P, Lindsey JC, Hughes MD, et al. Antiretroviral treatment for children with peripartum nevirapine exposure. *N Engl J Med* **2010**; 363:1510–20.
- Palanee-Phillips T, Schwartz K, Brown ER, et al. Characteristics of women enrolled into a randomized clinical trial of dapivirine vaginal ring for HIV-1 prevention. *PLoS One* **2015**; 10:e0128857.
- Husnik MJ, Brown ER, Marzinke M, et al; MTN-020/ASPIRE Study Team. Implementation of a novel adherence monitoring strategy in a phase III, blinded, placebo-controlled, HIV-1 prevention clinical trial. *J Acquir Immune Defic Syndr* **2017**; 76:330–7.
- Riddler SA, Husnik M, Gorbach PM, et al. Long-term follow-up of HIV seroconverters in microbicide trials - rationale, study design, and challenges in MTN-015. *HIV Clin Trials* **2016**; 17:204–11.
- Organization WH. Consolidated guidelines on HIV prevention, diagnosis, treatment and care for key populations. Geneva, Switzerland: WHO Press, **2016**.
- Rhee SY, Gonzales MJ, Kantor R, Betts BJ, Ravela J, Shafer RW. Human immunodeficiency virus reverse transcriptase and protease sequence database. *Nucleic Acids Res* **2003**; 31:298–303.
- Grant RM, Liegler T, Defechereux P, et al. Drug resistance and plasma viral RNA level after ineffective use of oral pre-exposure prophylaxis in women. *AIDS* **2015**; 29:331–7.
- Chirwa LI, Johnson JA, Niska RW, et al. CD4(+) cell count, viral load, and drug resistance patterns among heterosexual breakthrough HIV infections in a study of oral preexposure prophylaxis. *AIDS* **2014**; 28:223–6.
- Riddler SA, Husnik M, Ramjee G, et al. HIV disease progression among women following seroconversion during a tenofovir-based HIV prevention trial. *PLoS One* **2017**; 12:e0178594.
- Basson AE, Ntsala M, Martinson N, et al. Development of phenotypic HIV-1 drug resistance after exposure to single-dose nevirapine. *J Acquir Immune Defic Syndr* **2008**; 49:538–43.
- Flys TS, Donnell D, Mwatha A, et al. Persistence of K103N-containing HIV-1 variants after single-dose nevirapine for prevention of HIV-1 mother-to-child transmission. *J Infect Dis* **2007**; 195:711–5.
- Flys TS, McConnell MS, Matovu F, et al. Nevirapine resistance in women and infants after first versus repeated use of single-dose nevirapine for prevention of HIV-1 vertical transmission. *J Infect Dis* **2008**; 198:465–9.
- Martinson NA, Morris L, Gray G, et al. Selection and persistence of viral resistance in HIV-infected children after exposure to single-dose nevirapine. *J Acquir Immune Defic Syndr* **2007**; 44:148–53.
- Palmer S, Boltz V, Martinson N, et al. Persistence of nevirapine-resistant HIV-1 in women after single-dose nevirapine therapy for prevention of maternal-to-fetal HIV-1 transmission. *Proc Natl Acad Sci USA* **2006**; 103:7094–9.
- Nel A, Haazen W, Nuttall J, Romano J, Rosenberg Z, van Niekerk N. A safety and pharmacokinetic trial assessing delivery of dapivirine from a vaginal ring in healthy women. *AIDS* **2014**; 28:1479–87.
- Parikh UM, Kiepiela P, Ganesh S, et al; MTN-009 Protocol Team; IPTc Taskforce. Prevalence of HIV-1 drug resistance among women screening for HIV prevention trials in KwaZulu-Natal, South Africa (MTN-009). *PLoS One* **2013**; 8:e59787.