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MAJOR ARTICLE

Tenofovir Gel for Prevention of Herpes Simplex Virus Type 2 Acquisition: Findings From the VOICE Trial

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Background. Genital infection with herpes simplex virus type 2 (HSV-2) is common and increases risk of human immunodeficiency virus (HIV) transmission and acquisition. Pericoital use of tenofovir (TFV) gel provided protection from HSV-2 acquisition in the CAPRISA 004 study.

Methods. We measured estimate of effect of vaginal TFV 1% gel in preventing HSV-2 acquisition among women in VOICE, randomized, double-blinded, placebo-controlled trial assessing daily use of oral and vaginal TFV for HIV-1 preexposure prophylaxis. The TFV level in plasma at the first quarterly visit was used as a measure of gel use.

Results. Of 566 participants at risk for HSV-2 acquisition, 532 (94%) had first-quarter plasma TFV and end-of-study HSV-2 serologic data available. Over a follow-up period of 501 person-years, 92 incident cases of HSV-2 acquisition occurred: 77 were in women with no TFV detected in plasma, and 15 occurred in women with TFV detected in plasma (incidence, 20.6 cases/100 person-years [95% confidence interval [CI], 16.2–25.7] vs 11.9 cases/100 person-years [95% CI, 6.6–19.6], respectively). TFV detection in plasma was associated with a trend toward a reduced risk of HSV-2 seroconversion, with an unadjusted hazard ratio (HR) of 0.59 (95% CI, .34–1.02; $P = .060$) and a HR adjusted for site, age, having ≥2 male sex partners in the past 3 months, use of hormonal contraception, having anal sex in the past 3 months, and HIV status of 0.60 (95% CI, .33–1.08; $P = .086$).

Conclusions. Detection of TFV in plasma among TFV gel users was associated with a trend toward a reduced risk of HSV-2 acquisition, after controlling for sexual behavior and HIV-1 acquisition.

Keywords. Herpes simplex virus; genital herpes; HIV-1; tenofovir; preexposure prophylaxis.

Infection with genital herpes simplex virus (HSV) increases the risk of human immunodeficiency virus (HIV) acquisition and transmission. HSV-2 is prevalent in areas with the highest incidence of HIV-1, especially sub-Saharan Africa [1]. Most genital herpes is asymptomatic or causes unrecognized infection. Although antiviral treatment improves symptoms and reduces the risk of sexual transmission, it does not eliminate genital HSV shedding, and no vaccine is available [2, 3].

Tenofovir (TFV), an adenine nucleotide analog reverse transcriptase inhibitor, has in vitro anti–HSV-2 activity, although the effective concentration required for 90% inhibition of HSV-2 is high [4, 5]. Daily oral TFV disoproxil fumarate (TDF) and TDF/emtricitabine (FTC) as preexposure prophylaxis for HIV reduced HSV-2 acquisition by 30% in HSV-2–serodiscordant couples in Kenya and Uganda [6]. Although daily oral TDF/FTC as HSV-2 preexposure prophylaxis did not reduce HSV-2 acquisition among men who have sex with men in the iPrEX study, it reduced the number of genital ulcers observed [7]. One approach to overcoming the high EC_{90} is to take advantage of the high levels of local TFV delivered with vaginal application, which results in vaginal concentrations 1000-fold higher than those achieved with oral TDF [8]. In CAPRISA 004, South African women randomly assigned to use vaginal TFV 1% gel as pericoital prophylaxis for HIV-1 reduced the risk of HSV-2 acquisition by 51%, with higher efficacy among women who used TFV gel most frequently [9].

VOICE was a large, randomized, placebo-controlled trial of oral TDF, oral TDF-FTC, and vaginal TFV 1% gel as HIV-1 preexposure prophylaxis in reproductive-aged women in South Africa, Uganda, and Zimbabwe. Participants were asked to use study products daily. However, adherence to study products was low, and no regimen significantly reduced the risk of HIV-1 acquisition, in a modified intention-to-treat analysis [10]. In secondary analysis, women who were adherent to the use of TFV gel, defined by plasma TFV detection, had a lower likelihood of HIV acquisition [11]. We assessed whether the subgroup of women

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adherent to TFV gel use in the VOICE trial had a reduced rate of HSV-2 acquisition relative to those who were not adherent.

METHODS

Study Population and Procedures

The study protocol is available in the Supplementary Materials. The association between study product use and HSV-2 acquisition was evaluated using a prespecified exploratory analysis, and participants provided written informed consent. From September 2009 through June 2011, 12 320 women were screened at 15 sites in South Africa, Uganda, and Zimbabwe for enrollment into the VOICE study, as described elsewhere [10]. Briefly, we enrolled women aged 18–45 years who were neither pregnant nor breastfeeding; had normal renal, hematologic, and hepatic function; reported recent vaginal intercourse; and were using effective contraception. Participants were randomly assigned in a 1:1:1:1:1 ratio to one of 5 arms: oral TDF 300 mg and TDF-FTC placebo; oral TDF-FTC 300 mg/200 mg and TDF placebo; oral TDF placebo and oral TDF-FTC placebo; vaginal TFV 1% gel; and vaginal placebo gel [12]. Participants were counseled to use products daily. All participants were tested for sexually transmitted pathogens at enrollment, annually, and when indicated. Testing for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* was performed using a strand displacement amplification assay (BD ProbeTec; Becton Dickinson). Testing for *T. vaginalis* was performed using the OSOM Rapid Trichomonas Test (Genzyme). Syphilis testing was performed using a rapid plasma reagin screening test, followed by a confirmatory microhemagglutination assay for *Treponema pallidum* detection (MHA-TP) or a *T. pallidum* hemagglutination assay for reactive samples. Standard HIV risk-reduction counseling, individualized adherence counseling, condoms, and hepatitis B immunization were provided. Study product was withheld because of pregnancy, breastfeeding, and clinical or laboratory adverse events. All institutional review and ethics committees approved the study annually.

Study End Points

The primary end point was HSV-2 infection, measured by seroconversion, using the approach depicted in Figure 1. Plasma samples collected at the enrollment visit from all participants were screened for existing HSV-2 infection, using a HSV-2–specific enzyme immunoassay (EIA; Focus Diagnostics, Cypress, CA), to identify women susceptible to HSV-2 infection (defined by index values <3.5) [13]. This threshold was chosen to maximize the sensitivity of the assay to detect susceptible participants. Samples collected from susceptible participants during the visit after use of the study product ended were tested to assess whether seroconversion had occurred during study follow-up. HSV-2 serologic testing was also performed on stored plasma samples collected at quarterly visits from HSV-2 seroconverters, to better estimate the timing of HSV-2 infection. The assays were performed at BARC-SA, Global Central Laboratories (Johannesburg, South Africa), or at Magee-Womens Research Institute (Pittsburgh, PA); all results were reviewed for quality control at Magee-Womens Research Institute.

For the secondary outcome of HSV-2 acquisition confirmed by positive results of an HSV-2–specific Western blot (WB), participants identified as susceptible at study enrollment by the EIA also underwent WB of plasma specimens collected from the enrollment visit and the visit after the end of study product use. WB was performed at the University of Washington (Seattle, WA). Participants whose enrollment WB confirmed HSV-2 susceptibility and whose end-visit WB indicated acquisition of HSV-2–specific antibodies were defined as having WB-confirmed acquisition of HSV-2 infection during the study.

Drug-Level Analyses

Plasma TFV concentrations were determined using a validated liquid chromatography–tandem mass spectrometry method. The limit of TFV quantification was 0.31 ng/mL [14].

Statistical Analyses

For analysis of plasma TFV, we attempted to obtain at least 1 plasma test result from each of the 566 participants in the TFV gel arm who were HSV-2 EIA negative at baseline. For plasma pharmacokinetics (PK) analysis, most participants had only a single plasma sample tested for TFV, with this sample collected closest to the first quarterly (ie, month 3) follow-up visit. We considered only the first plasma sample per participant collected between months 2 and 6 of follow-up for analysis of product adherence; this first sample was highly correlated with the likelihood of subsequent plasma TFV detection and so is a reasonable surrogate of adherence throughout study participation [11]. A participant was dichotomously classified as having drug detected in plasma if TFV concentrations exceeded the lower limits of quantification stated above.

The primary analysis included only end points deemed as HSV-2 infections acquired after enrollment, as defined by EIA results. With EIA, incident HSV-2 infection was defined as a negative HSV-2 EIA result (index < 3.5) at baseline and a positive result (index ≥ 3.5) at the follow-up visit. For secondary analysis, WB results were classified as indeterminate, negative, or positive, and incident HSV-2 infection was defined as a negative HSV-2 WB result at baseline and a positive result at the follow-up visit. Since stored plasma samples collected at quarterly visit were tested by EIA for HSV-2 seroconversion, person-years used to calculate incidence rates for the primary analysis were counted from the baseline EIA result to the time of the last negative EIA result for those uninfected with HSV-2 and from the baseline EIA result to the midpoint between the last negative EIA result and the first positive follow-up EIA result for those infected with HSV-2 at study end. For secondary analysis, person-years were counted from the baseline WB result to the

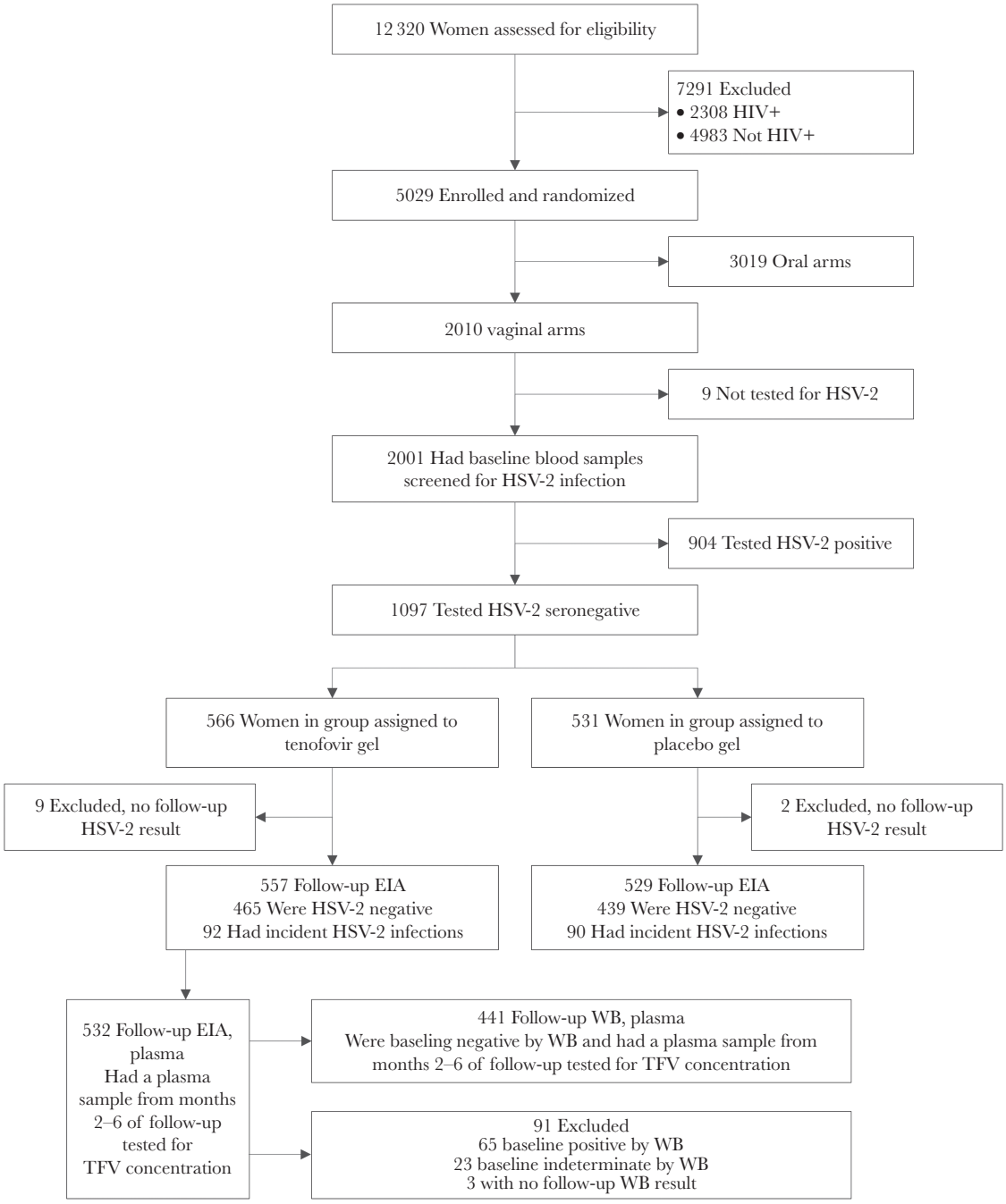

Figure 1. Enrollment and flow of women through the study. The most common reason for exclusion from the parent study was prevalent human immunodeficiency virus type 1 (HIV-1) infection (32% of excluded women [2308 of 7291]). Failure to complete screening and enrollment in the required 56-day window resulted in exclusion of 21% of excluded women, and abnormal laboratory results resulted in exclusion of 16%. Conditions related to reproductive outcomes resulted in the exclusion of 9%: 5.9% were pregnant at screening, with the remainder currently breast-feeding or intending to become pregnant in the next 2 years. Twenty-two participants had HIV-1 RNA detected by polymerase chain reaction assay in the enrollment plasma specimen and were excluded on the basis of acute HIV-1 infection. The 566 women assigned to the tenofovir (TFV) gel arm composed the population for this analysis. Of these, 532 had both end-of-study HSV-2 enzyme immunoassay (EIA) results and plasma TFV levels available to determine the primary end point (HSV-2 seroconversion defined by this assay, as related to detection of plasma TFV). Of these 532, 441 fulfilled criteria for inclusion in the secondary end point analysis, namely, HSV-2 seroconversion detected by HSV-2–specific Western blot (WB).

time of the negative follow-up WB result for those uninfected with HSV-2 and from the baseline WB result to the midpoint between the baseline WB result and the first positive follow-up WB result for those infected with HSV-2 at study end.

Logistic regression was used to determine predictors of HSV-2 prevalence at baseline. Cox proportional hazards regression was used to assess baseline characteristics and on-study HIV status (a time-varying parameter) associated with HSV-2 acquisition. For

the primary analysis, the effect of TFV gel on HSV-2 acquisition was estimated using a similar Cox regression model adjusted for participant characteristics, such as site, age, number of male sex partners, and HIV status (all chosen a priori); hormonal contraceptive use (injectable agents and oral pills); and anal sex (based on multivariable logistic regression model of predictors). For secondary analysis, the effect of TFV gel on WB-confirmed HSV-2 acquisition was estimated using Poisson regression with the natural log of follow-up time as the offset and adjustment for the same factors as the primary analysis.

Neither the estimated hazard ratio (HR) from Cox regression nor incidence rate ratio from Poisson regression, with or without adjustment for covariates, can be the interpreted as a causal estimate of effect attributable to TFV gel. For this association analysis, we followed a strategy for evaluating the level of residual confounding [11] by comparing the incidence of HSV-2 acquisition among participants randomly assigned to the placebo gel arm to that among the subset of participants randomly assigned to the TFV gel arm who had no TFV detected in plasma. Analyses were conducted using SAS (version 9.4; SAS Institute). All *P* values are 2-sided.

RESULTS

Characteristics of Study Population and Baseline HSV-2 Infection Status

Among 1004 participants randomly assigned to receive TFV gel and with baseline HSV EIA serologic results, 438 (44%) were HSV-2 seropositive and 566 (56%) were seronegative.

Characteristics of women in the TFV gel and placebo gel arms are presented by baseline HSV-2 infection status at enrollment in Table 1. For participants in the TFV gel arm, higher education level and oral contraceptive use were associated with a lower odds of HSV-2 infection; after controlling for age, income earning, number of male sex partners, and report of anal sex, only a trend toward a significant decrease remained for oral contraception use. Older age and report of anal sex were independently associated with an increased odds of HSV-2 infection. We also observed a trend toward a significantly increased odds of infection among participants reporting ≥2 male sex partners ($P = .06$). Number of live births was collinear with age and not entered in the multivariable model (Table 2).

Incidence of HSV-2 Seroconversion and Associated Risk Factors

Of 566 participants who were HSV-2 seronegative at enrollment, 532 (94%) had a plasma PK measurement and end-of-study HSV-2 EIA results available, and 529 (93%) had a vaginal swab PK measurement and end-of-study HSV-2 EIA results available (Figure 1). Overall follow-up comprised 501 person-years, with an end-of-study retention rate of 91%. During this time, 92 EIA-confirmed HSV-2 seroconversions occurred, for an overall incidence rate of 18.4 seroconversions/100 person-years. Characteristics associated with HSV-2 seroconversion are shown in Table 3, with the number of live births and report of ≥2 male sex partners showing a trend toward a significant association with HSV-2 seroconversion. In the modified

Table 1. Characteristics of Participants Randomized to Tenofovir (TFV) Gel or Placebo Gel Arms, by Results of Baseline Herpes Simplex Virus Type 2 (HSV-2)–Specific Serologic Testing

		TFV Gel Arm, HSV-2 Test Result		Placebo Gel Arm, HSV-2 Test Result	
Characteristic	All Participants[a] (n = 2001)	Positive (n = 438)	Negative (n = 566)	Positive (n = 466)	Negative (n = 531)
Age, y					
Mean ± SD	25.3 ± 5.2	26.9 ± 5.5	24.1 ± 4.6	26.7 ± 5.1	24.1 ± 4.8
Median (range)	24 (18–40)	26 (18–40)	23 (18–38)	26 (18–40)	23 (18–39)
Some secondary school education or higher	1834 (92)	385 (88)	532 (94)	418 (90)	499 (94)
Earns own income	1157 (58)	287 (66)	300 (53)	303 (65)	267 (50)
Live births, no., mean ± SD	1.5 ± 1.1	1.8 ± 1.2	1.2 ± 1.0	1.7 ± 1.2	1.4 ± 1.1
Currently married	425 (21)	86 (20)	124 (22)	90 (19)	125 (24)
≥2 male sex partners in past 3 mo	415 (21)	111 (25)	105 (19)	102 (22)	97 (18)
No. of sex acts in past 7 d, median (mean)	2.6 (3.3)	2.6 (3.3)	2.5 (3.8)	3.0 (3.3)	2.3 (2.5)
Condom use during last vaginal sex	1496 (75)	341 (78)	426 (75)	342 (74)	387 (73)
Anal sex, past 3 mo[b]	354 (18)	95 (22)	84 (15)	80 (18)	95 (18)
Contraception method					
Injectable	1425 (71)	312 (71)	392 (69)	337 (72)	384 (72)
Oral pills	452 (23)	91 (21)	147 (26)	104 (22)	110 (21)
Chlamydia trachomatis positivity	242 (12)	58 (13)	56 (10)	51 (11)	77 (15)
Neisseria gonorrhoeae positivity	60 (3)	13 (3)	11 (2)	18 (4)	18 (3)
Trichomonas vaginalis positivity	113 (6)	33 (8)	29 (5)	28 (6)	23 (4)
Treponema pallidum seropositivity	25 (1)	9 (2)	5 (1)	6 (1)	5 (1)
Bacterial vaginosis	788 (40)	186 (43)	210 (37)	188 (40)	204 (39)
Incident HIV infection	131 (7)	31 (7)	30 (5)	40 (9)	30 (6)

[a]The denominator is 2001 for all characteristics except some secondary school education or higher (n = 1998), earns own income (n = 2000), ≥2 male sex partners in past 3 mo (n = 1983), no. of sex acts in past 7 d (n = 1828), condom use during last vaginal sex (n = 1999), anal sex in past 3 mo (n = 1974), and bacterial vaginosis (n = 1991).

Table 2. Results of Multivariable Logistic Regression Analysis of the Association Between Characteristics of 987 Tenofovir Gel Recipients and Herpes Simplex Virus Type 2 Infection at Study Enrollment

Characteristic	Univariate OR (95% CI)	Multivariable OR (95% CI)[a]	*P*
Age	1.14 (1.11–1.17)	1.14 (1.11–1.18)	<.0001
Some secondary school education or higher	.48 (.31–.76)	.88 (.47–1.63)	.68
Earns own income	1.69 (1.30–2.18)	1.13 (.83–1.54)	.43
Number of live births	1.59 (1.41–1.80)	…	
Married	.87 (.64–1.19)	…	
Contraception method			
Injectable	.66 (.40–1.08)	.71 (.39–1.31)	.28
Oral pills	.51 (.30–.88)	.52 (.28–.99)	.05
≥2 male sex partners[b]	1.47 (1.09–1.99)	1.39 (.99–1.96)	.06
Condom use during last vaginal sex[b]	1.16 (.86–1.55)	…	
Anal sex[b]	1.59 (1.15–2.20)	1.58 (1.10–2.26)	.01

Analyses were adjusted by site.

Abbreviations: CI, confidence interval; OR, odds ratio.

[a]Characteristics were entered into the multivariable model if the univariate *P* value was ≤ .2 and they were not considered collinear with a stronger predictor (eg, number of live births was collinear with age).

[b]During the past 3 months.

intent-to-treat analysis, no difference in the incidence of HSV-2 seroconversion was observed between women randomly assigned to the TFV gel arm and those randomly assigned to the placebo arm (IRR, 0.96; 95% confidence interval [CI], .72–1.29; *P* = .80).

Effect of TFV Gel on HSV-2 Acquisition as Determined by EIA and Plasma PK Analysis

Incidence rates of HSV-2 acquisition among all 532 participants with plasma PK data are shown in Table 4, stratified by those with and those without TFV detected in plasma between months 2 and 6. Among these, 92 incident cases of HSV-2 infection occurred, and 130 (24%) participants had TFV detected in plasma. We observed a trend toward a lower rate of incident HSV-2 acquisitions among participants with TFV detected in plasma than among those without TFV detected, in both an unadjusted Cox regression model (HR, 0.59; 95% CI, .34–1.02; *P* = .06) and a similar model adjusted for site, age, ≥2 male sex partners, hormonal contraceptive use, anal sex, and HIV status (adjusted HR, 0.60; 95% CI, .33–1.08; *P* = .09).

Effect of TFV Gel on HSV-2 Acquisition With Confirmatory WB and Plasma PK Analysis

There were 47 incident cases of HSV-2 infection detected during follow-up by WB among the 441 participants in the TFV gel arm who had plasma PK data. Incidence rates are displayed in Table 4 overall and by detection or nondetection of TFV in plasma between months 2 and 6. The rate of incident HSV-2 infection detected by WB was statistically significantly lower among participants with TFV detected in plasma than

Table 3. Results of Multivariable Cox Proportional Hazards Regression Analysis of the Association Between Characteristics of 557 Tenofovir Gel Recipients and Acquisition of Herpes Simplex Virus Type 2 Infection During Study Participation

Characteristic	Univariate HR (95% CI)	Multivariable HR (95% CI)[a]	*P*
Age	.98 (.94–1.03)	…	
Some secondary school education or higher	1.12 (.49–2.58)	…	
Earns own income	1.10 (.73–1.67)	…	
Number of live births	1.14 (.95–1.37)	1.21 (.96–1.51)	.10
Married	.81 (.49–1.35)	…	
Contraception method			
Injectable	1.42 (.60–3.38)	…	
Oral pills	.81 (.32–2.06)	…	
≥2 male sex partners[b]	1.73 (1.11–2.72)	1.58 (.95–2.63)	.08
Condom use during last vaginal sex[b]	1.43 (.84–2.42)	1.31 (.76–2.26)	.33
Anal sex[b]	1.34 (.79–2.27)	…	
HIV infection	1.54 (.67–3.53)	…	

Analyses were adjusted by site.

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; HR, hazard ratio.

[a]Characteristics were entered into the multivariable model if the univariate *P* value was ≤.20.

[b]During the past 3 months.

Table 4. Incidence Rates of Herpes Simplex Virus Type 2 (HSV-2) Seroconversion Detected During Follow-up by Enzyme Immunoassay (EIA) and Western Blot (WB), by Tenofovir (TFV) Gel or Placebo Gel Receipt

Group	HSV-2 Negative at Baseline, No.	Incident HSV-2 Infection Detected During Follow-up, No. (%)	Time to HSV-2 Infection, Person-Years[a,b]	Incidence Rate, Cases/100 Person-Years (95% CI)
Participants with EIA data				
TFV gel arm				
Overall	532	92 (17.3)	500.9	18.4 (14.8–22.5)
TFV detected in plasma	130	15 (11.5)	126.4	11.9 (6.6–19.6)
No TFV detected in plasma	402	77 (19.2)	374.4	20.6 (16.2–25.7)
Placebo gel arm	529	90 (17.0)	508.1	17.7 (14.2–21.8)
Participants with WB data				
TFV gel arm				
Overall	441	47 (10.7)	440.3	10.7 (7.8–14.2)
TFV detected in plasma	110	4 (3.6)	114.2	3.5 (1.0–9.0)
No TFV detected in plasma	331	43 (13.0)	326.1	13.2 (9.5–17.8)
Placebo gel arm	441	43 (9.8)	436.1	9.9 (7.1–13.3)

[a]For participants with EIA data, person-years were counted from the baseline EIA result to the time of the last negative EIA result for those uninfected with HSV-2 and from the baseline EIA result to the midpoint between the last negative EIA result and the first positive follow-up EIA result for those infected with HSV-2 at study end.

[b]For participants with WB data, person-years were counted from the baseline WB result to the time of the negative follow-up WB result for those uninfected with HSV-2 and from the baseline WB result to the midpoint between the baseline WB result and the first positive follow-up WB result for those infected with HSV-2 at study end.

among those without TFV detected in plasma, in an unadjusted Poisson regression model, and a trend toward significance remained in a similar model adjusted for site, age, ≥2 male sex partners, hormonal contraceptive use, anal sex, and HIV status (adjusted IRR, 0.37; 95% CI, .13–1.08; $P = .07$).

Effects of HIV Acquisition on the Incidence of HSV-2 Acquisition Incidence and Relationship to TFV Detection

Of the 532 HSV-2–susceptible participants defined above, 25 acquired HIV-1 on or before HSV-2 seroconversion or, if still HSV-2 negative, by the end of follow-up. The incidence of HSV-2 acquisition among women who also acquired HIV-1 was not different from that among women who remained negative for HIV-1 (24.2 and 18.1 cases/100 person-years, respectively; IRR, 1.34; 95% CI, .59–3.06; $P = .49$), based on an unadjusted Poisson model. To assess whether concomitant HIV acquisition might have modified the effect of TFV gel on HSV-2 acquisition, we removed HIV seroconverters from the analysis assessing this relationship. Among the remaining 507 participants, the IRR in an adjusted Poisson model among participants with versus those without plasma TFV detected was similar to the IRR shown in Table 4 (IRR, 0.61; 95% CI, .35–1.06; $P = .08$). Similar results were seen for the analysis using vaginal swab PK data (data not shown). When these analyses were restricted to participants with Western blot results (n = 418 nonseroconverters), the association between plasma TFV detection and the incidence of HSV-2 acquisition trended toward significance (IRR, 0.37; 95% CI, .13–1.09; $P = .07$).

Comparison of Study Outcomes Among Participants Randomly Assigned to Receive TFV Gel Versus Placebo Gel

A potential concern in this analysis' approach is that the main outcome is compared among participants randomly assigned to receive the same intervention (daily use of TFV gel) and relies on detection of study product in plasma specimens obtained during participation. While such measurement can reliably categorize participants by product use, it cannot determine whether participants who actually used the study product were similar in other important respects to those who did not use it. If such differences were also associated with the likelihood of HSV-2 acquisition, they could be wholly or partly responsible for an apparent protective effect of TFV gel use. Examples of such characteristics include sexual behaviors, age, and marital status, all of which were significantly associated with the risk of HIV-1 acquisition in the parent study.

To address this concern, the incidence of HSV-2 acquisition was compared among participants randomly assigned to the placebo gel arm (90 of 529 women [508 person-years]; 17.7 cases/100 person-years) with that among the subset of participants randomly assigned to the TFV gel arm who had no TFV detected in plasma (77 of 402 women [374 person-years]; 20.6 cases/100 person-years). We reasoned that differences in HSV-2 acquisition would be minimal between participants randomly assigned to use TFV gel but least likely to be adherent and placebo users if TFV gel itself were primarily responsible for any protective effect. Indeed, the IRR adjusted for the factors specified above for incident HSV-2 infection (detected by EIA) between these 2 groups was 1.14 (95% CI, .83–1.55; $P = .42$). Similarly, the adjusted IRR for HSV-2 infection detected by WB between these groups was 1.28 (95% CI, .83–1.98; $P = .26$).

Effect of Oral TDF and TDF/FTC Use on HSV-2 Acquisition, as Determined by EIA and Plasma PK Analysis

Although not the focus of our primary analysis, given the limited availability of plasma TFV results from susceptible

participants (see below), we used a similar approach to assess whether women randomly assigned and adherent to the active oral product arms experienced protection from HSV-2 acquisition. Among all 2015 participants randomly assigned to these active oral product arms, 168 incident cases of HSV-2 infection (16.0%) occurred among 1049 participants with negative EIA results at baseline, and of 375 participants (35.7%) with available TFV plasma measurements between 2 and 6 months after enrollment, 131 (34.9%) had TFV detected in plasma, while 244 had no TFV detected. The rate of incident cases of HSV-2 infection detected by EIA was significantly lower among participants with TFV detected in plasma than in those without TFV detected, in an unadjusted Poisson regression model (10.5 cases/100 person-years [95% CI, 5.9–17.3] vs 19.5 cases/100 person-years [95% CI, 14.4–25.8]; unadjusted IRR, 0.54 [95% CI, .30–.96]), but did not remain so in a similar model adjusted for site, age, ≥2 male sex partners, hormonal contraceptive use, anal sex, and HIV status (adjusted IRR, 0.83; 95% CI, .45–1.55; $P = .56$). In a comparison of the incidence of HSV-2 acquisition between TFV gel recipients with TFV detected and active oral product recipients with TFV detected, no difference was observed in adjusted analyses (IRR, 0.68; 95% CI, .30–1.57; $P = .37$).

DISCUSSION

In this large prospective study of tenofovir-based products for HIV-1 preexposure prophylaxis in reproductive-aged women in 3 countries in sub-Saharan Africa, nearly half of the participants were already infected with HSV-2 at study entry, and almost 1 in 5 acquired HSV-2 infection over a median participation duration of 1 year. As expected, both prevalent and incident HSV-2 infection were independently associated with an increased risk of HIV-1 acquisition. In the intent-to-treat analysis, the incidence of HSV-2 acquisition among women randomly assigned to use vaginal TFV 1% gel daily did not differ from that among women randomly assigned to daily use of vaginal placebo gel. However, as previously reported, adherence to study products was low, especially among younger, unmarried women, who had the highest incidence of HIV-1 and HSV-2 acquisition. In this secondary analysis of women randomly assigned to use TFV gel, we used plasma measurement of TFV to stratify participants with evidence of TFV gel use as compared to those without such evidence. With this approach, detection of TFV in plasma was associated with a 40% reduction in HSV-2 acquisition.

Our finding of reduced HSV-2 acquisition among women who used TFV vaginal gel aligns with those from the CAPRISA 004 study, in which South African women randomly assigned to use vaginal TFV 1% gel as pericoital prophylaxis for HIV-1 experienced a reduced risk of HSV-2 acquisition of 51%, with a higher estimate of the effect among the most adherent women (71% reduction with >6 applications/month, based on the number of empty applicators returned) [9]. While both studies used the same TFV gel product, our study differs from the CAPRISA 004 study in several important ways. First, VOICE participants were asked to use vaginal TFV gel daily; CAPRISA 004 tested periocoital dosing (40 mg ≤12 hours before and 40 mg ≤12 after vaginal intercourse, with no more than 2 doses in a 24-hour period). Second, in VOICE, we had TFV test results for plasma samples obtained quarterly throughout follow-up as a systemic marker of vaginal application. Third, our diagnostic testing approach differed in that we confirmed all HSV-2 seroconversions by WB, which has a sensitivity and specificity of >99%. Finally, our finding of a 17% reduction in HSV-2 acquisition among women adherent to the active oral products (TDF and TDF/FTC) is similar to that reported among heterosexual couples who also used these products for preexposure prophylaxis in the Partners in Prevention Study [4] and would likely have achieved statistical significance in the adjusted analysis if TFV plasma results had been available for >36% of those at risk for infection at enrollment.

The protection against HSV-2 acquisition that we observed in women with evidence of TFV gel use is supported by biologic evidence for TFV's effect on HSV-2. In vitro, HSV-2 is suppressed at TFV levels of 101–200 μg/mL [4]. Vaginal application of TFV 1% gel achieves TFV-diphosphate concentrations that are on average >130-fold higher in vaginal tissue, relative to those achieved by oral dosing; TFV tissue concentrations are sustained at >100 μg/mL 24 hours after a single vaginal dose [8, 14]. These are substantially above the half maximal effective concentration for HSV-2.

Our findings have important limitations. First, they were derived from a secondary analysis based on a subset of participants in a randomized trial. Thus, potential bias could have introduced confounding into the observed association. Most critical, participants who were likely to use the study product (and thus to have detectable plasma TFV) were at lower risk of acquiring HSV-2, independent of TFV gel use. We attempted to mitigate this risk by controlling for numerous factors associated with acquisition of sexually transmitted infections, including number of male sex partners, age, and HIV-1 acquisition. We also found that the incidence of HSV-2 acquisition in participants randomly assigned to the placebo gel arm was not different than participants who were randomly assigned to the TFV gel arm yet had no evidence of TFV gel use, as assessed by plasma TFV measurement. Second, our ability to assess the association between TFV gel use and HSV-2 acquisition was limited by our inability to precisely measure the timing of these events. The estimates of timing for both TFV exposure and HSV-2 seroconversion are necessarily approximate because of the study's design and methods for timed plasma collection during follow-up. That said, our finding of concordant results between 2 temporal measurements of HSV-2 acquisition end points is reassuring. Third, collection of vaginal swab samples

was too infrequent (every 6 months or at any pelvic examination to assess symptoms) to allow for precise estimate of vaginal TFV detection relative to HSV-2 acquisition.

HSV-2 infection in reproductive-aged women is a major health concern and is especially problematic for women also at risk for sexual HIV-1 acquisition. A vaccine for HSV-2 will not likely be available soon. Our data add to evidence supporting the effectiveness of vaginal TFV gel in reducing women's risk of HSV-2 acquisition. With our finding of a similar protective effect of TFV gel against HIV-1 infection, using an analogous PK-based analysis [11], these data support further study of the delivery of TFV or derivatives to cervicovaginal tissue as a potential means of slowing the HSV-2 epidemic. Delivery systems that provide alternatives to gel use are under study, including vaginal rings that release TFV, TDF, or TFV alafenamide. Some alternatives are modified for codelivery of multiple active pharmaceutical ingredients, including antiretrovirals and contraceptive hormones. Access to such interventions could slow the combined trajectories of HSV-2 and HIV-1 acquisition in women.

Supplementary Data

Supplementary materials are available at The *Journal of Infectious Diseases online*. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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

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