

Effectiveness and Safety of Tenofovir Gel, an Antiretroviral Microbicide, for the Prevention of HIV Infection in Women

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The Centre for the AIDS Program of Research in South Africa (CAPRISA) 004 trial assessed the effectiveness and safety of a 1% vaginal gel formulation of tenofovir, a nucleotide reverse transcriptase inhibitor, for the prevention of HIV acquisition in women. A double-blind, randomized controlled trial was conducted comparing tenofovir gel ($n = 445$ women) with placebo gel ($n = 444$ women) in sexually active, HIV-uninfected 18- to 40-year-old women in urban and rural KwaZulu-Natal, South Africa. HIV serostatus, safety, sexual behavior, and gel and condom use were assessed at monthly follow-up visits for 30 months. HIV incidence in the tenofovir gel arm was 5.6 per 100 women-years (person time of study observation) (38 out of 680.6 women-years) compared with 9.1 per 100 women-years (60 out of 660.7 women-years) in the placebo gel arm (incidence rate ratio = 0.61; $P = 0.017$). In high adherers (gel adherence > 80%), HIV incidence was 54% lower ($P = 0.025$) in the tenofovir gel arm. In intermediate adherers (gel adherence 50 to 80%) and low adherers (gel adherence < 50%), the HIV incidence reduction was 38 and 28%, respectively. Tenofovir gel reduced HIV acquisition by an estimated 39% overall, and by 54% in women with high gel adherence. No increase in the overall adverse event rates was observed. There were no changes in viral load and no tenofovir resistance in HIV seroconverters. Tenofovir gel could potentially fill an important HIV prevention gap, especially for women unable to successfully negotiate mutual monogamy or condom use.

Women are disproportionately affected by the Acquired Immunodeficiency Syndrome (AIDS) epidemic in Africa, the region that accounts for 70% of global burden of Human Immunodeficiency Virus (HIV) infection (1). Current HIV prevention behavioral messages on abstinence, faithfulness, and condom promotion have had limited impact on HIV incidence rates in women, especially in sub-Saharan Africa, where young women bear the greatest HIV burden (2). The search for new technologies to prevent sexually transmitted HIV infection over the past three decades has had limited success. Only five of 37 randomized controlled trials, which tested 39 HIV prevention strategies, have demonstrated protection against sexual transmission of HIV infection (3). The successful trials tested medical male circumcision in South Africa (4), Kenya (5), and Uganda (6) (combined effective-

ness in reducing HIV acquisition was 57%), sexually transmitted infection (STI) treatment in Tanzania (effectiveness in reducing HIV acquisition was 42%) (7), and a HIV vaccine combination in Thailand (effectiveness in reducing HIV acquisition was 31%) (8). Hence, HIV prevention technologies that women can use and control remain a pressing priority (9).

Microbicides are products that can be applied to the vagina or rectum with the intention of reducing the acquisition of STIs, including HIV. An effective microbicide has the potential to alter the trajectory of the global HIV pandemic (10). Over the last 20 years of microbicide research, none of the 11 effectiveness trials of six candidate products have demonstrated meaningful protection against HIV infection (11).

Tenofovir, an adenosine nucleotide analog with potent activity against retroviruses (12), was initially developed and tested as a prophylactic in monkeys and was subsequently formulated for oral use as tenofovir disoproxil fumarate (Viread), which is now widely used for HIV treatment. Tenofovir's efficacy in suppressing viral replication, favorable safety profile, and long half-life (13) made it an ideal choice as the first antiretroviral drug to be formulated as a microbicide gel. In vitro and in vivo assessments of the 1% concentration of tenofovir in a gel formulation have demonstrated its potential as a microbicide (13). Tenofovir has shown efficacy against viral

challenge in animal models when administered as pre- or post-exposure prophylaxis (14, 15). In monkey challenge studies, tenofovir gel has shown protection with intermittent dosing and with a single pre-exposure dose (16). In early-stage clinical trials, tenofovir gel was well tolerated in both HIV-negative and HIV-positive women (17), with both daily and coitally related use of the gel being found to be acceptable and safe (18).

The purpose of this study was to assess the effectiveness and safety of tenofovir gel for the prevention of HIV infection in women.

Study design and population. Centre for the AIDS Program of Research in South Africa (CAPRISA) 004, a two-arm, double-blind, randomized, placebo-controlled trial, was conducted from May 2007 to March 2010. Women were enrolled at an urban and a rural clinic in KwaZulu-Natal, South Africa, but the study was not designed to assess the effectiveness of tenofovir in each clinic separately. Urban women were enrolled at the CAPRISA eThekweni Research Clinic, which is adjacent to an STI clinic located in the Durban city center. Rural women were enrolled at the CAPRISA Vulindlela Research Clinic adjacent to a comprehensive primary health care clinic in Vulindlela, which is a rural community of approximately 90,000 people and about 150 km northwest of Durban. Before the CAPRISA 004 trial, feasibility studies were conducted in order to assess HIV incidence and sexual behavior at both sites. Extrapolated HIV incidence rates from prevalence studies in the urban (19) and rural (20) sites were 15.6 and 11.2%, respectively. Reported anal sex rates were substantially lower at these two sites than we had observed in previous microbicide trials (21) in female sex workers in this region. Data from these feasibility studies were used as the basis for selecting these sites for the trial, as well as for the design and sample size calculations for the CAPRISA 004 trial.

HIV-negative women, from 18 to 40 years old, who were sexually active (defined as having engaged in vaginal sex at least twice in the 30 days before screening), not pregnant, and using a nonbarrier form of contraceptive were eligible for enrollment. Participants who had a history of adverse reactions to latex, planned to either travel away from the study site for more than 30 consecutive days, relocate away from the study site, become pregnant, or enroll in any other behavioral or investigational product study were excluded. Participants who had a creatinine clearance of <50 ml/min (22), had evidence of genital deep epithelial disruption, had in the past year participated in any research related to any vaginally applied product or products, or had an untreated STI or reproductive tract infection were also excluded. Women who met eligibility criteria and demonstrated adequate understanding of the trial (through a comprehension checklist) were enrolled after providing written informed consent. From May 2007 to January 2009, 2160 women were screened and 1085 were enrolled, of whom

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889 were included in the analysis (Fig. 1). Further information on the enrollment process and exclusions can be found in (23).

Enrolled women were randomly assigned in equal proportions to one of two study arms: tenofovir gel or placebo gel. Tenofovir gel comprised 40 mg of 9-(R)-2-phosphonomethoxypropyladenine monohydrate (PMPA) in a solution of purified water with edetate disodium, citric acid, glycerin, methylparaben, propylparaben, and hydroxyethylcellulose (HEC). The placebo gel was the “universal” HEC placebo gel, which has been shown to have minimal anti-HIV activity (24). Tenofovir and placebo gels appeared identical and were dispensed in the same pre-filled vaginal applicators with identical packaging.

A coitally related dosing strategy was selected to achieve high adherence on the basis of in-depth consultations with the communities involved. Sexual behavior data showed that women in the key study population had infrequent high-risk sex with migrant partners. Monkey challenge data and perinatal transmission studies informed the timing

of doses in relation to sex. The “before and after” sex doses were modeled on the timing of nevirapine in its proven strategy for preventing mother-to-child HIV transmission (25). Women were requested to insert one dose of gel within 12 hours before sex and a second dose of gel as soon as possible within 12 hours after sex and no more than two doses of gel in a 24-hour period. Hence, the dosing strategy is referred to as “BAT24.” The latter restriction was imposed because of the lack of human safety data on more than two doses of gel per day.

Gel adherence was defined as the estimated proportion of reported sex acts covered by two gel doses and calculated for each woman by dividing half the number of returned used applicators each month by the number of reported sex acts that month. Applicators that were not returned were regarded as unused for the purposes of calculating adherence. When we conducted a sensitivity analysis treating unreturned applicators as used, the results did not change materially. The median of each woman’s monthly adherence es-

timates was assigned as her overall gel adherence. This approach assumed that every reported sex act used two doses of gel. Although this assumption was not always applicable, adjusting for multiple sex acts within 24 hours made no material difference.

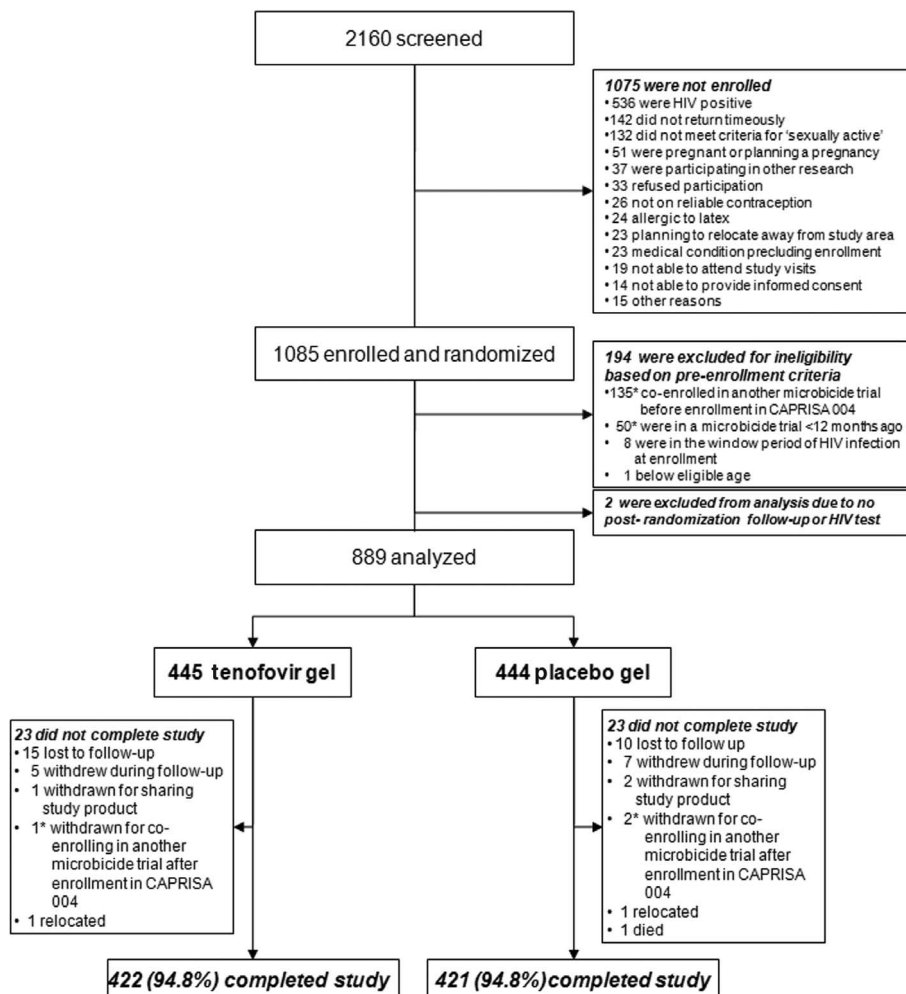
At enrollment and monthly follow-up visits, participants were provided with comprehensive HIV prevention services (HIV pre- and post-test counseling, HIV risk reduction counseling, condoms, and STI treatment), reproductive health services, and assigned study gel.

Participants were requested to return their used (from October 2007 onward) and unused applicators at every visit. Each month, the applicators returned by women as used and unused were counted, reconciled against the number dispensed, and thereafter discarded, in accordance with standard requirements for medical waste.

A comprehensive adherence support program assisted participants with the mechanics of applicator use, timing and dosing, avoidance of gel sharing, and incorporation of gel use into their daily routines. From October 2008, individualized motivational interviewing (26, 27) was introduced to assist participants so as to overcome obstacles to gel use and set goals for optimal adherence in the upcoming month. This included individualized adherence support and counseling, customized on the previous month’s experience of gel use, which was provided throughout the study. The women in this study were specifically and repeatedly counseled to only use the gel vaginally, and the lack of safety with rectal use was highlighted.

Each participant had monthly HIV and urine pregnancy testing [QuickVue One-Step hCG Urine Test (Quidel Corporation, San Diego, California)] performed before gel was dispensed. Because of a lack of pregnancy safety data, gel use was temporarily discontinued after a positive pregnancy test and resumed when the pregnancy test returned to negative. Self-reported data on gel use and sexual frequency during the last 30 days were collected at monthly visits, together with gel and condom use on the day of the last sex act, by means of a brief interviewer-administered questionnaire. Two months after study exit, participants attended a posttrial visit to assess HIV status and safety after product withdrawal.

Drug safety was assessed at every study visit by evaluating, grading, and recording adverse events experienced by participants. Participants underwent quarterly pelvic examinations and, if needed, colposcopy. Serology was performed for hepatitis B virus [Abbott Architect C8200 (Abbott Laboratories, Detroit, Michigan)] and herpes simplex type 2 virus [Kalon Enzyme Immunoassay (Kalon Biologicals, Ashgate, UK)]. Hematological, hepatic, and renal abnormalities were assessed at study months 3, 12, and 24; additionally when clinically indicated; and at study exit. Adverse events were graded for severity via the Division of AIDS Table for Grading Adult and Pediatric Adverse Events, 2004. Product use was temporarily discontinued for an adverse event at the discretion of the



*Note: co-enrollment occurred only in the urban clinic

Fig. 1. Screening, enrollment, randomization, and follow-up of the study participants.

study clinician. The trial (NCT00441298) was approved by the University of KwaZulu-Natal's Biomedical Research Ethics Committee (E111/06), Family Health International (FHI)'s Protection of Human Subjects Committee (9946), and the South African Medicines Control Council (20060835).

HIV, viral load, and genotypic resistance assays. Two HIV rapid tests, Determine HIV 1/2 (Abbott Laboratories, Chicago, Illinois) and Uni-Gold Recombigen® HIV test (Trinity Biotech, Wicklow, Ireland), were performed at each study visit. Participants with concordantly positive, discordant, or indeterminate results were assessed for possible seroconversion by means of two separate RNA polymerase chain reaction (PCR) [Roche Cobas TaqMan HIV-1 Monitor v1.0 (Roche Diagnostics, Branchburg, New Jersey)] assays, about 1 week apart. When HIV seroconversion was established, product use was discontinued, and women were referred to local AIDS treatment services, including the CAPRISA AIDS Treatment Program, which provides free antiretroviral therapy. Stored plasma, available from prior study visits by each seroconverter, was tested by means of RNA PCR so as to identify the window period for HIV infection (RNA PCR–positive but rapid HIV test–negative) at prior visits. By protocol, only eligible enrolled women with HIV infection during study follow-up, as confirmed by two independent RNA PCR results, were defined as HIV endpoints. Participants in the HIV window period at study exit were included as HIV endpoints if seroposi-

tivity was confirmed after the study. Thus, HIV infections were categorized as follows: (i) HIV endpoints, (ii) HIV infections not meeting the protocol definition for an HIV endpoint (did not have the two independent RNA PCR tests), (iii) window period HIV infections at enrollment (infected before study entry), (iv) posttrial HIV infections (infected after study exit), and (v) HIV infections among women who were enrolled and later found to be ineligible (23).

Tenofovir resistance testing and Western Blot [Genetics systems HIV-1 Western Blot kit (Bio-Rad Laboratories, Hercules, California)] were performed at the post-seroconversion visit. The HIV-1 *pol* gene was population sequenced by means of a certified (28) in-house assay. Viral RNA was extracted, and a 1.7-kb fragment spanning the *pol* gene was amplified by means of nested PCR with the Expand Long Template PCR System (Roche Diagnostics), as described previously (28). PCR products were sequenced (codons 1 to 99 of protease and codons 1 to 350 of reverse transcriptase) by using a BigDye Terminator v3.1 cycle sequencing kit and an ABI 3130XL DNA sequencer (Applied Biosystems, Foster City, California). Consensus sequences were aligned and manually edited by using the Sequencher version 4.5 program (GeneCodes, Ann Arbor, Michigan) and submitted to the Stanford University HIV Drug Resistance Database (<http://hivdb.stanford.edu>) to identify mutations.

Statistical analyses. In this endpoint-driven trial, participants were followed until 92 HIV

infections were observed, providing 90% power to detect a 50% effect (two-sided $\alpha = 0.05$). Originally, the study was designed with 80% power. Before their first data review, the Data Safety and Monitoring Board (DSMB) ratified a change to 90% power adjusted for two preplanned interim reviews, with stringent stopping guidelines.

Upon enrollment, a participant was assigned a sequential identification number, which corresponded to a unique envelope (accessible only to each study site pharmacist) that allocated her randomly, by using permuted block randomization of sizes 12 and 18, with no stratification, to one of six codes. The three codes assigned randomly to each of tenofovir and placebo gels were held in confidence by the product manufacturer and independent DSMB statistician.

The primary intent-to-treat analysis was based on a log-rank test, stratified by site. Duration of time on study was calculated from randomization to estimated date of HIV infection or date of withdrawal, whichever occurred first. A Poisson distribution was assumed for confidence intervals (CIs) of incidence rates and incidence rate ratios (IRRs). Fisher's exact test and the unpaired *t* test/Wilcoxon two-sample test were performed where appropriate. Proportional hazards regression models were used to calculate hazard ratios while adjusting for potentially important covariates. All reported *P* values are two-sided, and all CIs are 95%. The statistical analysis was performed using SAS (SAS Institute, Cary, North Carolina) version 9.1.3.

Table 1. Baseline demographic characteristics, sexual history, and contraceptive use by study participants in the CAPRISA 004 tenofovir gel trial.

	Site			Study arms		
	Rural (<i>n</i> = 611 women)	Urban (<i>n</i> = 278 women)	<i>P</i> value	Tenofovir (<i>n</i> = 445 women)	Placebo (<i>n</i> = 444 women)	<i>P</i> value
Demographic characteristics						
Mean age (years)	23.3	25.1	<0.001	24.2	23.6	0.131
Monthly income <1000 South African rand	86.1%	69.1%	<0.001	81.1%	80.4%	0.799
Married	6.5%	3.6%	0.085	5.8%	5.4%	0.884
Stable partner	77.0%	93.1%	<0.001	87.6%	88.5%	0.756
Sexual behavior						
Mean age sexual debut	17.3	17.7	0.014	17.4	17.4	0.782
Mean number sexual partners (lifetime)	2.1	6.0	<0.001	3.0	3.6	0.780
Mean age of oldest partner (past 30 days)	26.4	29.6	<0.001	27.7	27.1	0.299
Reported sex in the past 7 days	58.9%	68.3%	0.007	63.6%	60.1%	0.301
Always use condom during sex	22.9%	42.8%	<0.001	28.8%	29.5%	0.825
New partner (past 30 days)	0.5%	2.5%	0.014	1.3%	0.9%	0.753
Anal sex (past 30 days)	0.5%	0.4%	1.000	0.4%	0.5%	1.000
HSV-2 prevalence	47.6%	59.6%	0.001	53.5%	49.2%	0.202
Contraception						
Injectable	83.1	79.9	0.606*	80.7%	83.6%	0.288*
Oral	14.6	17.6		16.4%	14.6%	
Tubal ligation	2.1	2.5		2.9%	1.6%	
Hysterectomy	0.2	0.0		0	0.2%	

**P* value applicable to comparison for all forms of contraception.

Results. A total of 611 rural and 278 urban women met eligibility criteria, were enrolled, and followed up, for a total of 1341 women-years (mean = 18 months) and an overall study re-

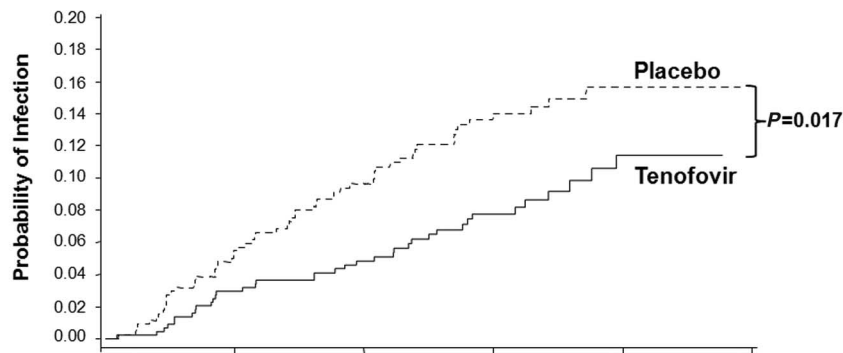
tention rate of 94.8%. Rural women were younger and poorer, with fewer lifetime sexual partners, and had lower sexual frequency and lower condom use (Table 1). At enrollment, there were no

significant differences in the demographic characteristics and sexual behavior of women in the tenofovir ($n = 445$ women) and placebo ($n = 444$ women) gel arms (Table 1).

HIV incidence and effectiveness. The tenofovir and placebo gel arms had 38 and 60 HIV endpoints, respectively. The HIV incidence rate in the tenofovir gel arm was 5.6 per 100 women-years (CI, 4.0 and 7.7) compared with 9.1 per 100 women-years (CI, 6.9 and 11.7) in the placebo gel arm (IRR = 0.61; CI, 0.40 and 0.94; $P = 0.017$).

HIV infection trends (Fig. 2) show that the tenofovir gel effect was evident soon after initiation of gel use. The steadily declining HIV incidence rates in placebo gel arm were 11.2, 10.5, 10.2, 9.4, and 9.1 per 100 women-years after follow-up for 6, 12, 18, 24, and 30 months, respectively (Fig. 2). In contrast, the HIV incidence rate in the tenofovir gel arm remained in a narrow range between 5.2 and 6.0 per 100 women-years during the study. The HIV incidence rate in the tenofovir gel arm, when compared with the placebo gel arm, was 50% ($P = 0.007$) lower after 12 months of follow-up and 40% ($P = 0.013$) lower after 24 months of follow-up (Fig. 2).

After adjusting for baseline covariates including, age, site, anal sex history, contraceptive method, HSV-2 antibody status, and condom use, the hazard ratio was 0.63 (CI, 0.42 and 0.94; $P = 0.025$). All 98



Months of follow-up	6	12	18	24	30
Cumulative HIV endpoints	37	65	88	97	98
Cumulative women-years	432	833	1143	1305	1341
HIV incidence rates (Tenofovir vs Placebo)	6.0 vs 11.2	5.2 vs 10.5	5.3 vs 10.2	5.6 vs 10.2	5.6 vs 9.1
Effectiveness (P-value)	47% (0.064)	50% (0.007)	47% (0.004)	40% (0.013)	39% (0.017)

Fig. 2. Kaplan-Meier estimates of cumulative probability of HIV infection in the tenofovir and placebo gel arms. The table provides the cumulative number of HIV endpoints, corresponding HIV incidence rates, and effectiveness of tenofovir gel for each additional 6 months of follow-up.

Table 2. Impact of adherence and non-endpoint HIV infections on the effectiveness of tenofovir gel in HIV prevention in the CAPRISA 004 tenofovir gel trial.

	No. of HIV infections/women years		N	HIV incidence		Incidence rate ratio	Effectiveness	95% CI	P value
	Tenofovir	Placebo		Tenofovir gel (95% CI)	Placebo gel (95% CI)				
Overall effectiveness of tenofovir gel									
HIV endpoints	38/680.6	60/660.7	889	5.6 (4.0, 7.7)	9.1 (6.9, 11.7)	0.61	39%	6, 60	0.017
Site-specific effectiveness									
Rural	25/484.7	42/461.2	611	5.2 (3.3, 7.6)	9.1 (6.6, 12.3)	0.57	43%	5, 67	0.023
Urban	13/195.9	18/199.5	278	6.6 (3.5, 11.3)	9.0 (5.3, 14.3)	0.74	26%	-59, 67	0.380
HIV endpoints by levels of adherence*									
High adherers (>80% gel adherence)	11/259.2	25/269.4	336	4.2 (2.1, 7.6)	9.3 (6.0, 13.7)	0.46	54%	4, 80	0.025
Intermediate adherers (50–80% adherence)	10/159.8	10/99.7	181	6.3 (3.0, 11.5)	10.0 (4.8, 18.4)	0.62	38%	-67, 77	0.343
Low adherers (<50% gel adherence)	16/258.5	25/290.6	367	6.2 (3.5, 10.1)	8.6 (5.6, 12.7)	0.72	28%	-40, 64	0.303
Sensitivity analyses									
HIV endpoints plus HIV infection not meeting protocol definition	39/680.6	60/660.7	889	5.7 (4.1, 7.8)	9.1 (6.9, 11.7)	0.63	37%	4, 59	0.023
HIV endpoints plus ineligibly enrolled	40/720.1	63/698.6	1075	5.6 (4.0, 7.6)	9.0 (6.9, 11.5)	0.62	38%	7, 60	0.015
HIV endpoints plus women with post-trial infection	39/680.6	64/660.7	889	5.7 (4.1, 7.8)	9.7 (7.5, 12.4)	0.59	41%	11, 61	0.015
Per protocol analysis†	32/589.2	53/575.4	889	5.4 (3.7, 7.7)	9.2 (6.9, 12.0)	0.59	41%	7, 63	0.017
All HIV infections‡	43/720.1	76/698.8	1085	6.0 (4.3, 8.0)	10.9 (8.6, 13.6)	0.55	45%	19, 63	0.003
Adjusted analysis§	38	60	889		Hazard ratio = 0.63		37%	6, 58	0.025

*Adherence could not be calculated for the five women who reported no sex during their follow-up in the study. †Excludes all visits after 3 month's interruption of drug supply. ‡All HIV infections = protocol-defined HIV endpoints ($n = 98$ women) + HIV infection not meeting protocol definition ($n = 1$ woman who did not have 2 RNA PCR results) + HIV infections among ineligibly enrolled women ($n = 5$ women) + post-trial HIV infections ($n = 5$ women) + window period HIV infections in eligible women ($n = 8$ women) + window period HIV infections in ineligibly enrolled women ($n = 2$ women). §Adjusted for the following baseline covariates: age, site, parity, number of sexual partners (past 30 days), presence of STI, anal sex, contraceptive method, HSV-2 antibody status, and condom use; HSV-2 status is indeterminate in four women and missing in five women.

HIV endpoints were Western Blot–positive. One additional HIV infection did not meet the protocol endpoint requirement of two independent RNA PCR results. There were five HIV infections among ineligibly enrolled women, 10 window-period HIV infections (two among ineligibly enrolled women), and five posttrial HIV infections. Sensitivity analyses (Table 2), which include these additional HIV infections, do not differ appreciably from the overall 39% level of effectiveness.

Gel adherence and sexual behavior. Over the entire duration of the study, 181,340 applicators were dispensed, and 95.2% of these were returned. Each month, study participants returned an average of 6.0 used applicators and reported a mean of 5.0 sex acts. Coital frequency, gel adherence, and condom use during the trial were similar in the tenofovir and placebo gel arms. Gel acceptability was high; 97.4% of the study participants found the gel to be acceptable, and 97.9% indicated that they would use it if it prevented HIV.

Five women reported having no sex during follow-up in the study. Adherence estimates based on applicator returns for the remaining 884 women indicate that, on average, 72.2% (median = 60.2%) of self-reported sex acts in the last 30 days were covered by two doses of gel. In the 336 high gel adherers, HIV incidence was 54% lower (IRR = 0.46; CI, 0.20 and 0.94; $P = 0.025$) in the tenofovir gel arm (Table 2). In intermediate gel adherers and low gel adherers, the HIV incidence reduction was 38% ($P = 0.343$) and 28% ($P = 0.303$), respectively. The mean number of sex acts in the high, intermediate, and low gel adherers was 3.2, 5.0, and 6.7 per month, respectively.

Over the 30 months of follow-up, reported coital frequency declined steadily (Fig. 3), from 7.2 sex acts per month in the first 6 months to 3.1 sex acts per month in months 18 to 24 ($P < 0.001$). In women who did not acquire HIV, overall median gel adherence was 61.3%, increasing from 55.0% in the first 6 months to 75.0% in months 18 to 24 ($P < 0.001$). In HIV seroconverters, overall median adherence (until product discontinuation after HIV infection) was 59.2%, ranging from 56.9% in the first 6 months to 61.3% in months 18 to 24 ($P = 0.735$). Overall, condoms were reportedly used in 80.3% of sex acts, increasing from 78.5% in the first 6 months to 84.3% in months 18 to 24 ($P < 0.001$) (Fig. 3).

Safety and pregnancy outcomes. There were 4692 adverse events reported during the study, with 94.3% (838 out of 889) of the study participants reporting at least one adverse event. Adverse event rates were 3.55 per women-year in the tenofovir and 3.44 per women-year in the placebo gel arms ($P = 0.265$). Women in the tenofovir gel arm reported more instances of diarrhea (Table 3) than those using placebo gel (16.9 versus 11.0%, $P = 0.015$). There were 39 serious adverse events, including one death. In the 37 hepatitis B virus carriers (20 randomized to tenofovir gel and 17 to placebo gel), there were two cases of “hepatic flares” (alanine aminotransferase > 5 times the upper limit of normal) in each arm. Further infor-

mation on grading of the hepatic, renal, and bone-adverse events can be found in (23).

Five participants discontinued gel use for a total of 1.04 women-years because of adverse events; four were due to genital findings, and one was due to congestive cardiac failure.

The overall pregnancy rate was 4.0 per 100 women-years: 3.2 per 100 women-years in the tenofovir arm and 4.7 per 100 women-years in the placebo arm ($P = 0.183$) (Table 3). At the time of analysis, there were six ongoing pregnancies, and 58.3% of the remaining 48 pregnancies had resulted in a full-term live birth. There were no significant differences in pregnancy outcomes by study arm, and there were no congenital anomalies. A total of 20.9 women-years of follow-up occurred while women were not using gel because of pregnancy.

Viral load and tenofovir resistance. The mean log HIV viral load at the time when HIV seroconversion was identified was 4.65 [interquartile range (IQR), 4.04 to 5.39] and 4.30 (IQR, 3.56 to 5.17) log copies per milliliter in the tenofovir gel arm ($n = 38$ HIV seroconverters) and placebo gel arm ($n = 60$ HIV seroconverters), respectively ($P = 0.147$).

It is estimated that the HIV seroconverters were exposed to gel episodically for about 3 to 4 weeks after infection and the resistance assays ($n = 35$ HIV seroconverters) were performed on average 20 weeks after the estimated date of infection. There were no tenofovir-related resistance mutations (K65R or K70E) detected, and none of the women had thymidine analog mutations (M41L, L210W, T215Y/F, D67N, K70R, and K219Q/E) or mutations that confer multiple-nucleoside reverse transcriptase inhibitor resistance (29).

Discussion. Tenofovir gel reduced HIV infection by an estimated 39%. The protective ef-

fect of coitally related tenofovir gel use was evident soon after initiation and peaked at 50% after 12 months of gel use. This protective effect is evident irrespective of sexual behavior, condom use, herpes simplex type 2 virus infection, or urban/rural differences. A trend of higher effectiveness was observed as gel adherence improved; high adherers had a 54% lower HIV incidence rate in the tenofovir gel arm.

The observed level of effectiveness is dependent on both the efficacy of the product and the participants' willingness and ability to use it as prescribed. Inadequate adherence is the most serious challenge to obtaining an accurate estimate of product efficacy (30). To address this, we implemented an intensive adherence support program with motivational strategies that depended on reliable measurement of adherence. Monitoring of this key behavior in the trial included an objective count of used and unused applicators returned each month and did not rely solely on self-reported use. Despite this adherence program and high gel acceptability, about 40% of the women in this study had below 50% gel adherence. Future trials will need to place greater emphasis on enhancing and objectively measuring adherence, in light of its substantial influence on the trial outcome.

In this study population, women with the highest gel adherence tended to have the lowest reported coital frequency. Despite their lower coital frequency, these women had HIV incidence rates comparable (in the placebo gel arm) with those in women with much higher coital frequencies, highlighting the importance of infrequent but very high-risk sex with migrant men. The impact of coitally related tenofovir gel was substantial in this group, indicating its potential to alter the course of the HIV epidemic in southern Africa, where young women engaging in sex with

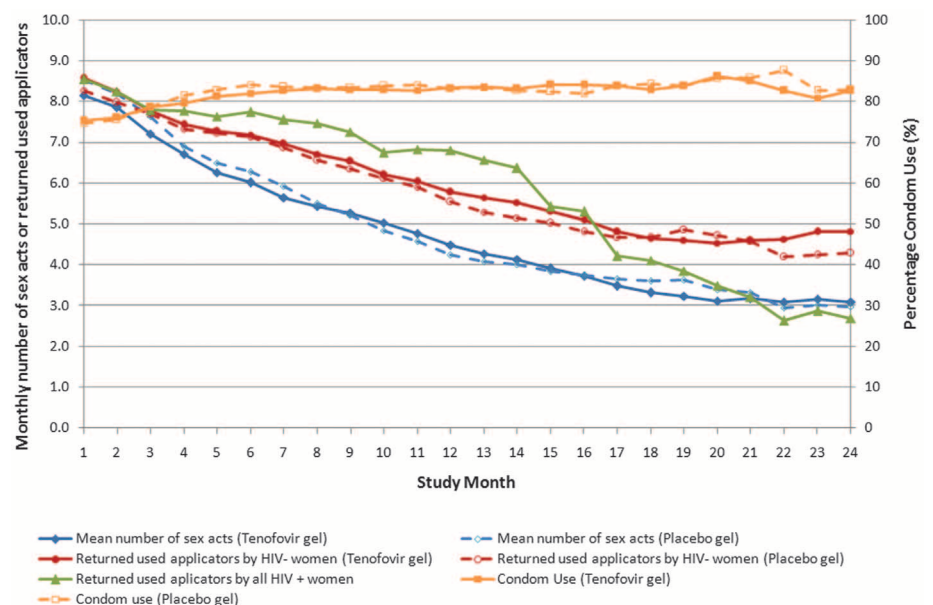


Fig. 3. Trends in coital frequency, condom use, and gel use (gel use by HIV status) in relation to duration of follow-up.

migrant men is the key driver in the spread of HIV infection (31). On a cautionary note, the effectiveness of coitally related tenofovir gel appeared to decline after 18 months; reasons for this are unclear, and factors, including the possibility of declining number of gel applications and/or adherence over time, need further investigation.

HIV incidence rates observed in this study population were high because KwaZulu-Natal province is at the epicenter of South Africa's "explosive" HIV epidemic (32). Although the women in the tenofovir gel arm had a substantially lower HIV incidence rate than that of the placebo arm, they still had an unacceptably high HIV incidence rate, consistently above 5 per 100 women-years. This highlights the need to seek higher levels of adherence and effectiveness with tenofovir gel and to develop other effective prevention strategies for use in combination with

tenofovir gel. Encouragingly, there was no evidence of risk compensation (33), in which individuals increase their HIV risk by reducing their use of proven prevention modalities, such as condoms, in favor of less effective or unproven prevention strategies. Instead, we observed declining HIV incidence rates in the placebo gel arm. This may have been due to their declining coital frequency and increasing condom use. However, the consistently high levels of self-reported condom use in the last sex act need to be interpreted cautiously because these may be affected by inaccurate recall and may be indicative of condom use in only the last sex act and not all sex acts.

We found no empirical evidence for the theoretical concern that tenofovir gel may mask HIV infection and that withdrawal of tenofovir gel use after study exit may lead to the unmasking of these infections.

Coitally related tenofovir gel use was safe. There were no increases in renal, hepatic, pregnancy-related, or genital-adverse events. The increased risk of diarrhea in women using tenofovir gel may possibly have been due to a local tenofovir effect; further investigation is needed to establish the mechanism for this observed adverse effect. The reported cases of diarrhea were mild and self-limiting, rarely requiring medication.

There was no renal toxicity—the most important tenofovir-related safety concern (34)—although the study excluded women with compromised creatinine clearance at enrollment. Increases in hepatic flares, which have been reported upon cessation of oral tenofovir use in hepatitis B-infected individuals (35), was not observed in this study, possibly because of the low systemic absorption of tenofovir from the gel formulation (17). No safety concerns were identified in the 22

Table 3. Adverse events and other safety markers in the CAPRISA 004 tenofovir gel trial.

	Tenofovir gel	Placebo gel	P value
	Events/participants/ (percent with ≥ 1 event)	Events/participants/ (percent with ≥ 1 event)	
Number of adverse events	2419/423/(95.1)	2273/415/(93.5)	0.32
Deaths	0/0(0)	1/1(0.2)	0.50
Serious adverse events:	23/21(4.7)	16/16(3.6)	0.50
total serious adverse events			
Pregnancy-related serious adverse events	8/8(1.8)	8/8(1.8)	1.00
Grade 3* adverse events	19/15(3.4)	18/16(3.6)	0.86
Grade 4* adverse events	4/4(0.9)	4/3(0.7)	1.00
Common adverse events			
Influenza	365/216(48.5)	314/220(49.5)	0.79
Vaginal discharge	203/156(35.1)	239/156(35.1)	1.00
Headache	126/93(20.9)	133/102(23.0)	0.53
Urinary tract infection	135/100(22.5)	120/93(20.9)	0.63
Diarrhea and gastrointestinal infections	91/75(16.9)	65/49(11.0)	0.02
Upper respiratory tract infections	162/114(25.6)	145/100(22.5)	0.31
Genital adverse events			
Disrupted epithelium, e.g., genital ulceration	18/18(4.0)	14/13(2.9)	0.47
Intact epithelium, e.g., erythema	48/41(9.2)	42/33(7.4)	0.40
Urogenital symptoms (such as menorrhagia)	312/210(47.2)	394/238(53.6)	0.06
Vaginal candidiasis	156/114(25.6)	187/130(29.3)	0.23
Other	182/131(29.4)	176/123(27.7)	0.60
Laboratory parameters: any abnormality after randomization			
Hepatic			
Aspartate aminotransferase (AST)	29/21(4.7)	36/29(6.5)	0.25
Alanine transaminase (ALT)	42/33(7.4)	50/40(9.0)	0.38
Renal			
Raised creatinine	3/3(0.7)	1/1(0.2)	0.62
Low potassium	119/95(21.3)	99/83(18.7)	0.36
Abnormal sodium	54/48(10.8)	43/41(9.2)	0.50
Hematological			
Anemia	52/34(7.6)	46/29(6.5)	0.60
Neutropenia	19/16(3.6)	13/11(2.5)	0.44
Bone			
Low phosphate	79/62(13.9)	65/51(11.5)	0.31
Abnormal calcium	16/15(3.4)	14/13(2.9)	0.85
Fractures	5/4(0.9)	2/2(0.5)	0.69
Pregnancy rate per 100 women-years	3.2	4.7	0.18
Proportion of pregnancies resulting in live births	66.7	51.9	0.38

*Grade 3 and 4 adverse events refer to the grading for severity according to the Division of AIDS Table for Grading Adult and Pediatric Adverse Events, 2004 (<http://rsc.tech-res.com/safetyandpharmacovigilance/>).

women exposed to tenofovir gel in early pregnancy, providing further evidence to support the analysis of the Antiretroviral Pregnancy Register (36), which showed no increases in congenital anomalies. No tenofovir-related resistance was found in the 35 women exposed to tenofovir gel early in acute HIV infection. Further studies to identify tenofovir resistance at earlier time points after infection, in both the genital and systemic compartments, are needed. Coitally related tenofovir gel use showed no impact on viral load in HIV seroconverters.

This test-of-concept study had several limitations; the relatively small sample size and the small number of study sites restrict the broad generalizability of the results. The study's adherence program needed to attain higher and sustained levels of adherence. The co-enrollment challenge was a setback at the urban site. It did not, however, affect the estimated effectiveness of tenofovir gel when infections in co-enrolled women were included in the analysis. It is not possible to derive from this study any conclusions on the safety and effectiveness of tenofovir gel for anal sex. Similarly, it is not possible to make any conclusions on the effectiveness of tenofovir gel in relation to the timing of gel applications because when gel was applied, BAT24 was usually followed.

Currently, there are five large-scale trials assessing oral pre-exposure prophylaxis with tenofovir or tenofovir-emtricitabine (37) in men who have sex with men, intravenous drug users, and heterosexual men and women. One of these, the Microbicide Trials Network (MTN) 003 trial (38), is assessing the effectiveness of daily tenofovir gel for HIV prevention. This critically important study will provide urgently needed evidence on whether more frequent dosing can improve adherence and effectiveness of tenofovir gel without compromising safety. Additional studies are needed to corroborate the findings of the CAPRISA 004 trial and to assess the safety, effectiveness, adherence, and cost advantages or disadvantages of coitally related tenofovir gel as compared with daily tenofovir in either the gel or oral formulation for HIV prevention in women.

Conclusion. Coitally related tenofovir gel appears safe and effective in preventing HIV infection. Once these promising findings have been corroborated, this antiretroviral microbicide could potentially fill an important HIV prevention gap, especially for women unable to successfully negotiate mutual monogamy or condom use.

References and Notes

- UNAIDS, WHO, *AIDS Epidemic Update* (Joint United Nations Programme on HIV/AIDS and World Health Organization, Geneva, 2009).
- S. S. Abdoool Karim, G. J. Churchyard, Q. Abdoool Karim, S. D. Lawn, *Lancet* **374**, 921 (2009).
- N. S. Padian, S. I. McCoy, J. E. Balkus, J. N. Wasserheit, *AIDS* **24**, 621 (2010).
- B. Auvert *et al.*, *PLoS Med.* **2**, e298 (2005).
- R. C. Bailey *et al.*, *Lancet* **369**, 643 (2007).
- R. H. Gray *et al.*, *Lancet* **369**, 657 (2007).
- H. Grosskurth *et al.*, *Lancet* **346**, 530 (1995).

- S. Reerks-Ngarm *et al.*; MOPH-TAVEG Investigators, *N. Engl. J. Med.* **361**, 2209 (2009).
- Z. A. Stein, *Am. J. Public Health* **80**, 460 (1990).
- C. Watts, P. Vickerman, *AIDS* **15**, 543 (2001).
- S. S. Abdoool Karim, C. Baxter, *HIV Ther.* **3**, 3 (2009).
- E. De Clercq, *Biochem. Pharmacol.* **73**, 911 (2007).
- L. C. Rohan *et al.*, *PLoS ONE* **5**, e9310 (2010).
- R. A. Otten *et al.*, *J. Virol.* **74**, 9771 (2000).
- C.-C. Tsai *et al.*, *Science* **270**, 1197 (1995).
- U.-M. Parikh *et al.*, *J. Virol.* **83**, 10358 (2009).
- K. H. Mayer *et al.*; HPTN 050 Protocol Team, *AIDS* **20**, 543 (2006).
- S. L. Hillier, paper presented at the Microbicide 2008, New Delhi, India, 2008.
- A. B. Kharsany, Q. A. Karim, S. S. Abdoool Karim, *AIDS Care* **22**, 533 (2010).
- A. B. Kharsany *et al.*, *HIV Med.*; published online 17 May 2010.
- L. Van Damme *et al.*; COL-1492 Study Group, *Lancet* **360**, 971 (2002).
- D. W. Cockcroft, M. H. Gault, *Nephron* **16**, 31 (1976).
- Materials and methods are available as supporting material on *Science Online*.
- D. Tien *et al.*, *AIDS Res. Hum. Retroviruses* **21**, 845 (2005).
- J. B. Jackson *et al.*, *Lancet* **362**, 859 (2003).
- R. A. Ferrer, K. M. Morrow, W. A. Fisher, J. D. Fisher, *AIDS Care* **22**, 997 (2010).
- W. R. Miller, S. Rollnick, *Motivational Interviewing* (Guilford, New York, 1991).
- V. Pillay *et al.*, *Antivir. Ther.* **13** (suppl. 2), 101 (2008).
- Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. In the mutants, other amino acids were substituted at certain locations; for example, K65R indicates that lysine at position 65 was replaced by arginine.
- S. W. Lagakos, A. R. Gable, Eds., *Methodological Challenges in Biomedical HIV Prevention Trials* (National Academy of Sciences, Washington, DC, 2008).
- M. N. Lurie *et al.*, *AIDS* **17**, 2245 (2003).
- Q. Abdoool-Karim, S. S. Abdoool-Karim, *Int. J. Epidemiol.* **31**, 37 (2002).
- M. M. Cassell, D. T. Halperin, J. D. Shelton, D. Stanton, *BMJ* **332**, 605 (2006).
- B. Schaaf, S. P. Aries, E. Kramme, J. Steinhoff, K. Dalhoff, *Clin. Infect. Dis.* **37**, e41 (2003).
- M. Crane *et al.*, *J. Infect. Dis.* **199**, 974 (2009).
- R. S. Brown, D. Goodwin, S. Zhang, E. Fagan, paper presented at the 13th International Symposium on Viral Hepatitis and Liver Disease (ISVHLD), Washington, DC, 20 to 24 March 2009.
- AVAC, Pre-Exposure Prophylaxis (PrEP), available at www.avac.org/ht/dl/sp/i/262/pid/262/cat_id/458/cids/453,458 (2010) (accessed 12 June 2010).
- Microbicide Trials Network, "Phase 2B safety and effectiveness study of tenofovir 1% gel, tenofovir disoproxil fumarate tablet, and emtricitabine/tenofovir disoproxil fumarate tablet for the prevention of HIV infection in women," available at www.mtnstopshiv.org/node/70 (2010) (accessed 12 June 2010).
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Supporting Online Material

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Materials and Methods

Table S1

References

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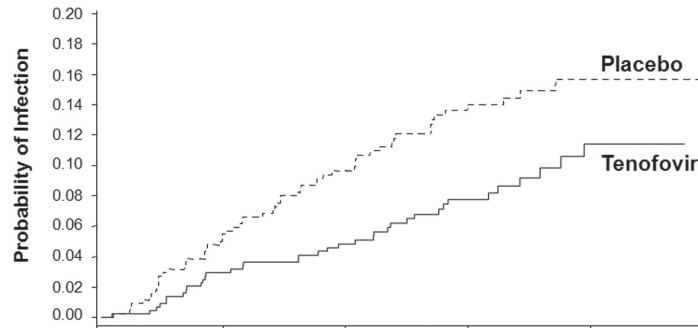
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ERRATUM

Post date 29 July 2011

Research Articles: “Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women” by Q. Abdool Karim *et al.* (3 September 2010, p. 1168). On page 1170, the first sentence of the first full paragraph should read, “Upon enrollment, a participant was assigned a sequential identification number, which corresponded to a unique envelope (accessible only to each study site pharmacist) that allocated her randomly, by using permuted block randomization of sizes 12 and 18, stratified by site, to one of six codes.” In Fig. 2, the *P* value at the end of the Placebo and Tenofovir lines in the top chart should be deleted; the corrected figure is below. The Fig. 2 caption should include, “The lines showing the cumulative probability of HIV infection provide data on the first 28 months of follow-up only.” In Table 2, an additional footnote should be cited at the end of the first-column entry “HIV endpoints plus HIV infection not meeting protocol definition” that reads, “This analysis is stipulated in the Trial’s Statistical Analysis Plan as primary.”



Months of follow-up	6	12	18	24	30
Cumulative HIV endpoints	37	65	88	97	98
Cumulative women-years	432	833	1143	1305	1341
HIV incidence rates (Tenofovir vs Placebo)	6.0 vs 11.2	5.2 vs 10.5	5.3 vs 10.2	5.6 vs 10.2	5.6 vs 9.1
Effectiveness (<i>P</i>-value)	47% (0.064)	50% (0.007)	47% (0.004)	40% (0.013)	39% (0.017)

Effectiveness and Safety of Tenofovir Gel, an Antiretroviral Microbicide, for the Prevention of HIV Infection in Women

Quarraisha Abdool Karim, Salim S. Abdool Karim, Janet A. Frohlich, Anneke C. Grobler, Cheryl Baxter, Leila E. Mansoor, Ayesha B. M. Kharsany, Sengeziwe Sibeko, Koleka P. Mlisana, Zaheen Omar, Tanuja N. Gengiah, Silvia Maarschalk, Natasha Arulappan, Mukelisiwe Mlotshwa, Lynn Morris, Douglas Taylor and on behalf of the CAPRISA 004 Trial Group

Science **329** (5996), 1168-1174.
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Vaginal Gel Versus HIV

HIV prevention technologies for women are urgently needed, especially in sub-Saharan Africa where young women bear the greatest burden of the HIV epidemic. **Abdool Karim et al.** (p. 1168; published online 19 July) present the results of the CAPRISA 004 randomized control trial. The nearly 3-year-long trial, conducted in urban and rural South African women, tested the efficacy of a vaginal gel containing the antiretroviral drug tenofovir in preventing HIV infection. The dosing strategy required application of the gel both before and after coitus, and with this regime HIV infection was reduced by approximately 39% overall, by 54% in women with high adherence to the protocol, and with no increase in overall adverse event rates.

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