ORIGINAL ARTICLE

Antiretroviral Preexposure Prophylaxis for Heterosexual HIV Transmission in Botswana

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ABSTRACT

BACKGROUND

Preexposure prophylaxis with antiretroviral agents has been shown to reduce the transmission of human immunodeficiency virus (HIV) among men who have sex with men; however, the efficacy among heterosexuals is uncertain.

METHODS

We randomly assigned HIV-seronegative men and women to receive either tenofovir disoproxil fumarate and emtricitabine (TDF–FTC) or matching placebo once daily. Monthly study visits were scheduled, and participants received a comprehensive package of prevention services, including HIV testing, counseling on adherence to medication, management of sexually transmitted infections, monitoring for adverse events, and individualized counseling on risk reduction; bone mineral density testing was performed semiannually in a subgroup of participants.

RESULTS

A total of 1219 men and women underwent randomization (45.7% women) and were followed for 1563 person-years (median, 1.1 years; maximum, 3.7 years). Because of low retention and logistic limitations, we concluded the study early and followed enrolled participants through an orderly study closure rather than expanding enrollment. The TDF–FTC group had higher rates of nausea (18.5% vs. 7.1%, P<0.001), vomiting (11.3% vs. 7.1%, P=0.008), and dizziness (15.1% vs. 11.0%, P=0.03) than the placebo group, but the rates of serious adverse events were similar (P=0.90). Participants who received TDF–FTC, as compared with those who received placebo, had a significant decline in bone mineral density. K65R, M184V, and A62V resistance mutations developed in 1 participant in the TDF–FTC group who had had an unrecognized acute HIV infection at enrollment. In a modified intention-to-treat analysis that included the 33 participants who became infected during the study (9 in the TDF–FTC group and 24 in the placebo group; 1.2 and 3.1 infections per 100 person-years, respectively), the efficacy of TDF–FTC was 62.2% (95% confidence interval, 21.5 to 83.4; P=0.03).

CONCLUSIONS

Daily TDF–FTC prophylaxis prevented HIV infection in sexually active heterosexual adults. The long-term safety of daily TDF–FTC prophylaxis, including the effect on bone mineral density, remains unknown. (Funded by the Centers for Disease Control and Prevention and the National Institutes of Health; TDF2 ClinicalTrials.gov number, NCT00448669.)

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Biomedical strategies to prevent sexual transmission of human immunodeficiency virus (HIV) remain limited.[1] In animal models, preexposure prophylaxis with tenofovir disoproxil fumarate (TDF) or with the combination of TDF and emtricitabine (TDF–FTC) can prevent infections with HIV or hybrid simian–human immunodeficiency virus after vaginal or rectal challenge.[2,3] In humans, daily preexposure prophylaxis with TDF–FTC has been shown to reduce transmission of HIV by 44% among men who have sex with men[4]; however, the findings from studies in heterosexual populations have been mixed.[5-8]

Botswana has the world's second highest prevalence of HIV infection, estimated in 2008 to be 17.6% overall and approximately 40% among adults 30 to 44 years of age.[9] Although Botswana was among the first African countries to introduce HIV prevention programs focused on male circumcision, prevention of mother-to-child transmission, and voluntary HIV counseling and testing, there is a need for additional prevention strategies to better control the generalized epidemic in this country. In this context, we conducted the TDF2 study to evaluate whether prophylaxis with daily oral TDF–FTC could prevent HIV infection among sexually active heterosexual adults.

Methods

Study Population and Design

In this phase 3, randomized, double-blind, placebo-controlled clinical trial, we screened for enrollment men and women in the Botswana cities of Francistown and Gaborone. Eligible participants were HIV-seronegative, sexually active adults, 18 to 39 years of age, with normal results on serum chemical and hematologic tests, a negative test result for hepatitis B virus surface antigen, and no chronic illnesses or long-term medication use. Female participants could not be pregnant or breast-feeding and had to be willing to use effective contraception to enroll in the study. Eligible men and women who provided written informed consent to participate in the study were randomly assigned, in a 1:1 ratio, to receive oral TDF–FTC or placebo once daily; randomization was performed with the use of random, permuted blocks of six, with stratification according to site and sex. For complete details of the study conduct, see the protocol, available with the full text of this article at NEJM.org.

TDF1 and TDF2 Studies

In 2005, the study investigators initiated the TDF1 study to evaluate the safety and efficacy of preexposure prophylaxis with TDF, as compared with placebo, with both study drugs administered once daily. When data from studies in animals later showed the superior efficacy of TDF–FTC,[3] we changed the active drug to TDF–FTC (TDF2 study). Beginning on February 20, 2007, a total of 18 participants in the TDF1 study enrolled in the TDF2 study and continued to receive the study medication as previously assigned (i.e., active drug or placebo). The first new participant in the TDF2 study was enrolled on March 22, 2007, and the last on October 23, 2009. Data from the TDF1 study are not included in this report.

During the study period, we observed a lower-than-expected rate of retention, owing primarily to relocation for work or school (i.e., early withdrawals) or to participants' scheduling conflicts leading to repeatedly missed study visits. Revised sample-size calculations indicated that we would need to expand enrollment to more than 2500 participants for the study to maintain at least 80% power to identify an efficacy result. Given this difficult logistical challenge, the sponsors and study investigators decided to proceed with an orderly conclusion to the study. Study investigators and participants remained unaware of the treatment assignments during this decision-making process. We informed participants about the impending closure of the study and allowed them to continue the study medication until protocol revisions were approved by the ethics committees in the United States and Botswana; exit procedures began on March 29, 2010. Study staff attempted to find all participants who were not in active follow-up as of this date (excluding study participants who had withdrawn) and to test them for HIV infection. Participants who missed three or more consecutive recent visits and could not be located at the end of the study were considered to have been permanently lost to follow-up. All participants exited the study by May 31, 2010, except for participants who had become infected with HIV, were pregnant, or had an ongoing serious adverse event at exit; we followed these partici-

pants for up to 1 additional year after discontinuation of the study medication. The last participant visit occurred on March 11, 2011.

STUDY OVERSIGHT

The Botswana Health Research and Development Committee and the institutional review board at the U.S. Centers for Disease Control and Prevention reviewed and approved the protocol, consent forms, and supporting documents. All participants provided written informed consent; as required by Botswana law, parental or guardian consent was obtained for participants 18 to 20 years of age. Participants could withdraw informed consent and exit the study at any time. We asked exiting participants to complete all exit procedures and contacted them again for study procedures only if the results of laboratory tests at exit were abnormal. An independent data and safety monitoring board (see the Supplementary Appendix, available at NEJM.org) reviewed safety data at least annually and identified no safety concerns that necessitated modifying or stopping the study. We planned a single interim efficacy analysis once half the expected HIV end points had occurred or half the total expected person-years of follow-up had been accrued. Since neither threshold had been reached when we decided to conclude the study, the data and safety monitoring board did not review the efficacy data. Gilead Sciences donated the study medication but was not involved in the collection or analysis of the data or the preparation of the manuscript. All the authors vouch for the completeness and accuracy of the data presented and for the fidelity of the study to the protocol.

STUDY PROCEDURES

After obtaining written consent, we screened potential participants by means of a brief interview and performed pregnancy testing on the women. We tested for HIV infection by means of dual, parallel, rapid HIV tests on whole-blood samples, using Determine HIV-1/2 (Abbott Diagnostics) and either Uni-Gold Recombigen HIV (Trinity Biotech) or OraQuick Advance HIV-1/2 (OraSure Technologies) (see Fig. S1 in the Supplementary Appendix). We then obtained whole-blood samples from the HIV-uninfected, nonpregnant participants who were deemed to be eligible after the screening interview and performed serum chemical and hematologic measurements and testing for hepatitis B virus surface antigen. We assessed the participants' understanding of the study using a computer-based education program; participants were required to receive a score of at least 80% on a comprehension test to be eligible for enrollment.

We assessed the sexual behavior and condom use of the enrolled participants by means of face-to-face interviews (at baseline and monthly thereafter) and by means of audio computer-assisted self-interviews (at baseline and semiannually thereafter) and provided a comprehensive package of HIV prevention services, including individualized counseling on risk reduction, free male and female condoms, and screening for sexually transmitted infections followed, if applicable, by partner notification and treatment. HIV rapid testing at enrollment and at subsequent monthly visits was performed with the use of the OraQuick Advance HIV-1/2 test of oral transudate (Fig. S2 in the Supplementary Appendix). We assessed bone mineral density by means of dual-energy x-ray absorptiometry (Hologic QDR 4500C) in a subgroup of eligible, consenting participants at enrollment and semi-annually thereafter.

Study visits were scheduled every 30 days until completion of the study, and participants were instructed to return to the clinic for evaluation in the event of an illness. Study staff contacted participants by telephone or visited them in their home after missed study visits. During monthly study visits, we performed testing for HIV infection and for pregnancy, collected information about any illness and side effects, assessed adherence to medication and self-reported sexual activity and condom use, and provided condoms. HIV-negative, nonpregnant participants with no contraindications (e.g., a new medical illness) were provided with a new bottle of study medication and given counseling on adherence. We collected any remaining medication from participants who were exiting the study. During quarterly visits, we performed serum chemistry testing and provided individualized counseling on risk reduction, with further discussion at intercurrent visits when requested by the participant. During semiannual visits, we performed physical examinations, including pelvic and genital examinations, and collected genital samples to test for sexually transmitted infections. At completion of the study, we tested all participants for HIV infection, using an enzyme-linked immunosorbent assay (ELISA).

Specific laboratory assessments are shown in Table S1 in the Supplementary Appendix.

For participants with positive or indeterminate results on HIV testing of oral transudate, we performed dual, parallel, fingerstick rapid HIV testing and provided counseling (Fig. S2 in the Supplementary Appendix). In addition, we obtained blood samples to test for HIV infection by means of ELISA and to measure the baseline viral load and CD4+ lymphocyte count; we also tested for antiretroviral resistance mutations, using standard and ultrasensitive techniques. We provided all results to HIV-infected participants and their designated health care providers. We retrospectively performed testing for HIV RNA on stored samples from participants who had undergone seroconversion in order to determine the earliest visit at which infection could be documented.

We measured the participants' plasma drug levels by means of an ultrahigh-performance liquid chromatography–mass spectrometry assay (lower limit of detection, 0.3 ng per milliliter for both tenofovir and emtricitabine). For each participant who underwent seroconversion, we assayed the available specimen collected before and closest to the interpolated seroconversion date and then randomly chose specimens obtained during the same study visit from three participants in the TDF–FTC group, matched for sex and study site, who had not undergone seroconversion. We limited testing to participants who reported having taken the study medication within the previous 30 days. Additional study procedures are described in the Supplementary Appendix.

STATISTICAL ANALYSIS

The primary efficacy end point was the difference in the rates of HIV infection between participants assigned to receive TDF–FTC and those assigned to receive placebo. The primary hypothesis was that TDF–FTC, as compared with placebo, would reduce the rate of HIV infection by at least 65%, with a predefined lower boundary for the 95% confidence interval of 10%. Assuming an annual incidence rate of HIV infection of 5%, we estimated that a sample of approximately 1000 participants overall would give the study more than 80% power, at a one-sided significance level of 0.05, to test the primary hypothesis and would detect at least a 65% reduction in the rate of HIV infection with TDF–FTC. To account for uncertainty in our estimated incidence rate of HIV infection and for losses to follow-up, the target for enrollment was 1200 participants.

The initial efficacy analysis included all study participants who were randomly assigned to receive a study medication (intention-to-treat cohort). The per-protocol primary efficacy analysis excluded participants who were retrospectively determined, by means of an RNA polymerase-chain-reaction assay, to have been infected with HIV at the time of enrollment (modified intention-to-treat cohort). We calculated efficacy using Cox regression to estimate the hazard rate and Kaplan–Meier methods to estimate the cumulative probability of HIV-1 infection. We also performed an as-treated analysis in which follow-up data from participants in the modified intention-to-treat cohort were censored 30 days after the participants reported taking their last dose of study medication.

Safety analyses were performed in the intention-to-treat cohort. Primary safety end points included the frequency of adverse clinical or laboratory events and the change in bone mineral density. We graded adverse events according to the National Institutes of Health Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (December 2004). All analyses were performed with the use of SAS software, version 9.2 (SAS Institute). Although one-sided tests were used for sample-size calculations, we used two-sided tests for all final analyses. P values of less than 0.05 were considered to indicate statistical significance.

RESULTS

STUDY PARTICIPANTS

We screened 2533 volunteers; 52.2% were eligible for enrollment. The primary reasons for ineligibility were abnormal laboratory test results (16.4%), especially hyperamylasemia (6.0%), hyperbilirubinemia (3.9%), and positivity for hepatitis B virus surface antigen (3.5%); not being sexually active (9.4%); and HIV infection (10.6%) (Fig. 1). A total of 1219 participants (45.7% women) underwent randomization (Fig. 1 and Table 1). Participants were followed for 1563 person-years (median, 1.1 years; maximum, 3.7 years). Among 1200 participants who were followed for seroconversion, 1072 (89.3%) completed exit procedures, with a final HIV test result available for 1070 (89.2%). However, 397 participants (33.1%) did not complete the study per protocol, of whom 115 (9.6%) were considered to

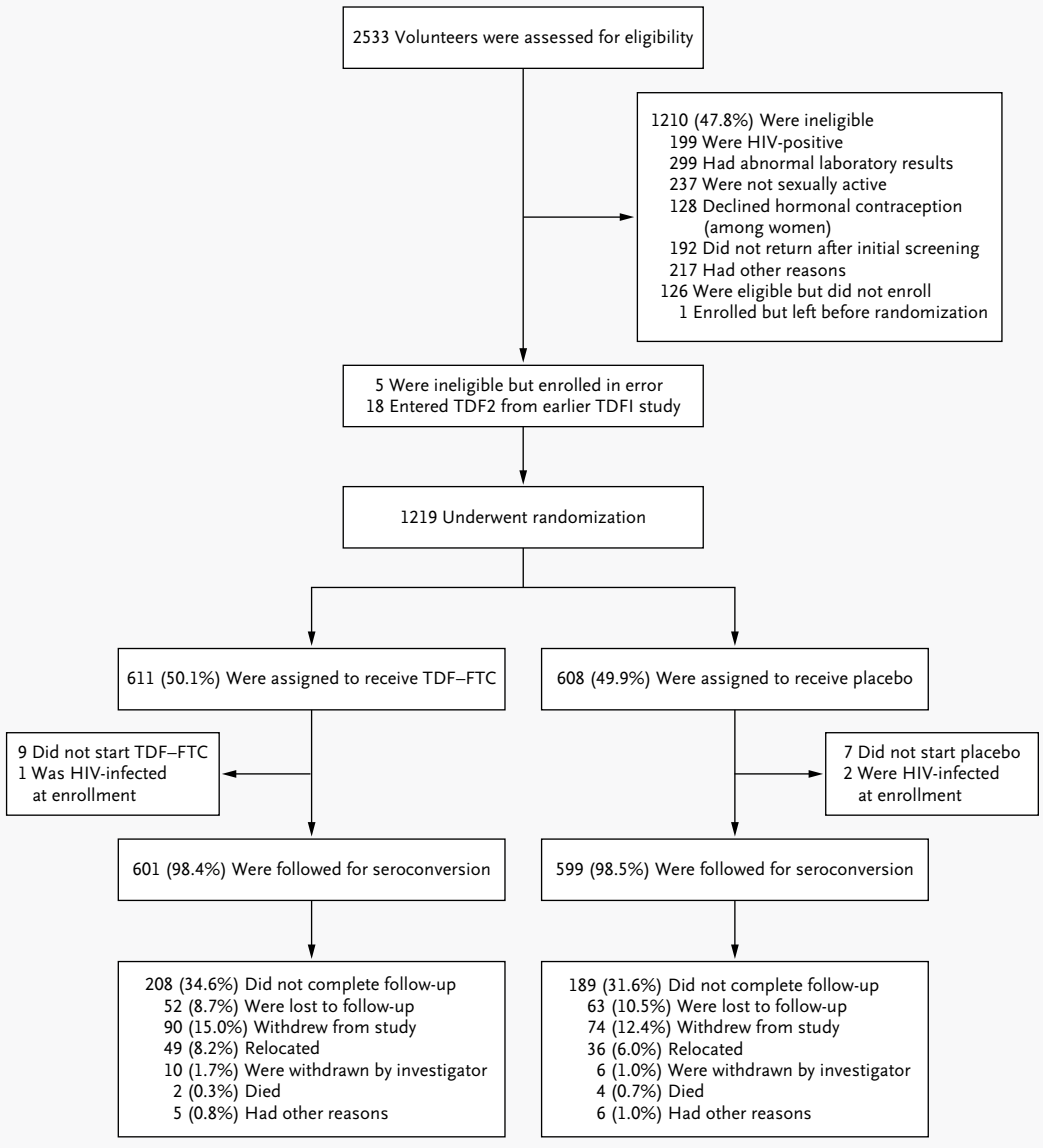

Figure 1. Screening, Enrollment, Randomization, and Follow-up.

Participants could have more than one reason for being ineligible. Reasons that potential participants were ineligible other than those listed specifically in the figure included that they did not pass the comprehension test (74 volunteers), were pregnant (43), were taking long-term medication (33), expected to be relocating soon (29), were breast-feeding (13), lived outside the study area (12), had a history of kidney or bone disease (4), were enrolled in another HIV prevention trial (2), declined to be tested for HIV infection (2), were unwilling to take the study medication (2), did not have parental consent (1), were too busy with work (1), and did not meet the age criteria (1). A total of 542 of 601 participants in the TDF–FTC group (90.2%) and 528 of 599 in the placebo group (88.1%) had known HIV status at study exit. HIV denotes human immunodeficiency virus, and TDF–FTC tenofovir disoproxil fumarate and emtricitabine.

have been permanently lost to follow-up (Fig. 1). The study groups did not differ significantly with respect to the rates of withdrawal (15.0% in the TDF–FTC group and 12.4% in the placebo group, $P=0.21$) or loss to follow-up (8.7% and 10.5%, respectively; $P=0.28$). When asked about their perceived treatment assignment, a similar percentage of participants in each group guessed that

they were receiving TDF–FTC (Table S2 in the Supplementary Appendix).

MEDICATION ADHERENCE AND RISK BEHAVIOR

The two groups had similar rates of adherence to the study medication, as estimated by means of pill counts (84.1% in the TDF–FTC group and 83.7% in the placebo group, P=0.79) and self-reported adherence for the preceding 3 days (94.4% and 94.1%, respectively; P=0.32). A total of 12 participants in the TDF–FTC group (2.0%) and 9 in the placebo group (1.5%) had their study medication permanently discontinued for safety reasons (P=0.66).

At the time of enrollment, most participants reported having had only one sexual partner in the preceding month (Table 1). The percentage of sexual episodes in which condoms were used with the main or most recent casual sexual partner was similar in the two study groups at enrollment (81.4% [range, 76.6 to 86.4] in the TDF–FTC group and 79.2% [range, 71.6 to 87.6] in the placebo group, P=0.66) and remained stable over time (Fig. S3 in the Supplementary Appendix), and the reported number of sexual partners declined similarly in both groups during the course of the study (Fig. S4 in the Supplementary Appendix). Few participants reported having had any anal intercourse (2.6% in the TDF–FTC group and 2.5% in the placebo group, P=1.00); none of these participants underwent seroconversion.

Table 1. Baseline Characteristics of the Study Participants.*

Characteristic	TDF–FTC (N=611)	Placebo (N=608)	P Value
Age — no. (%)			0.34
18–20 yr	10 (1.6)	15 (2.5)	
21–29 yr	550 (90.0)	532 (87.5)	
30–39 yr	51 (8.3)	61 (10.0)	
Sex — no. (%)			0.93
Female	280 (45.8)	277 (45.6)	
Male	331 (54.2)	331 (54.4)	
Educational level — no. (%)			1.00
Primary or less	20 (3.3)	20 (3.3)	
Secondary	446 (73.0)	445 (73.2)	
Postsecondary	145 (23.7)	143 (23.5)	
Marital status — no. (%)			0.45
Married	32 (5.2)	38 (6.2)	
Single	578 (94.6)	567 (93.3)	
Divorced or widowed	1 (0.2)	3 (0.5)	
City of residence — no. (%)			0.97
Gaborone	326 (53.4)	325 (53.5)	
Francistown	285 (46.6)	283 (46.5)	
Any alcohol use in the previous 3 mo — no./total no. (%)	359/601 (59.7)	340/599 (56.8)	0.30
Male circumcision — no./total no. (%)	41/330 (12.4)	39/328 (11.9)	0.83
Female hormonal contraception chosen at enrollment — no./total no. (%)			0.57
Oral contraceptive	158/280 (56.4)	171/277 (61.7)	
Injection or implant	105/280 (37.5)	90/277 (32.5)	
No contraception†	15/280 (5.4)	13/277 (4.7)	
Other method‡	2/280 (0.7)	3/277 (1.1)	

Table 1. (Continued.)

Characteristic	TDF–FTC (N=611)	Placebo (N=608)	P Value
Sexual behaviors — no. (%)			
No. of lifetime sex partners			0.07
1	28 (4.6)	20 (3.3)	
2–4	224 (36.7)	209 (34.4)	
5–9	198 (32.4)	181 (29.8)	
≥10	134 (21.9)	171 (28.1)	
Unknown	27 (4.4)	27 (4.4)	
No. of sex partners in previous mo			0.93
0	73 (11.9)	78 (12.8)	
1	410 (67.1)	405 (66.6)	
2	86 (14.1)	86 (14.1)	
≥3	32 (5.2)	28 (4.6)	
Unknown	10 (1.6)	11 (1.8)	
Sex with known HIV-positive partner in previous mo			0.85
Yes	21 (3.4)	22 (3.6)	
No	479 (78.4)	474 (78.0)	
Unknown	111 (18.2)	112 (18.4)	
Sexually transmitted infections — no./total no. (%)			
Herpes simplex virus 2 seropositivity	208/601 (34.6)	220/600 (36.7)	0.11
Neisseria gonorrhoeae	12/578 (2.1)	12/565 (2.1)	0.96
Chlamydia trachomatis	43/578 (7.4)	54/566 (9.5)	0.20
Treponema pallidum	5/599 (0.8)	9/597 (1.5)	0.28
Trichomonas vaginalis in women	19/256 (7.4)	14/248 (5.6)	0.42

* Percentages may not total 100 because of rounding. HIV denotes human immunodeficiency virus, and TDF–FTC tenofovir disoproxil fumarate and emtricitabine.

† Of the participants who were not using hormonal contraception at enrollment, 15 (9 in the TDF–FTC group and 6 in the placebo group) started hormonal contraception at the time they started the study medication or soon thereafter. Of the remaining 13 participants, 11 (6 in the TDF–FTC group and 5 in the placebo group) never started the study medication. The other 2 participants (both in the placebo group) did not receive hormonal contraception, owing to a shortage of contraceptives during their enrollment visit, and they did not return after their enrollment visit.

‡ Other contraceptive methods included intrauterine devices and bilateral tubal ligation.

SAFETY

Nausea, vomiting, and dizziness occurred more frequently among participants who received TDF–FTC than among those who received placebo (nausea: 18.5% vs. 7.1%, P<0.001; vomiting: 11.3% vs. 7.1%, P=0.008; and dizziness: 15.1% vs. 11.0%, P=0.03) (Table 2). These symptoms lessened after the first month (Fig. S5, S6, and S7 in the Supplementary Appendix). In contrast, leukorrhea and urethral discharge occurred more frequently among participants who received placebo (leukorrhea: 4.9% with TDF–FTC vs. 8.7% with placebo, P=0.01; and urethral discharge: 0.3% vs. 1.8%, P=0.03). Rates of chlamydial infection and gonorrhea were similar in the two groups (chlamydial infection: 12.4% with TDF–FTC and 12.3% with placebo, P=0.80; gonorrhea: 4.6% and 3.0%, respectively; P=0.10) (Table S3 in the Supplementary Appendix). There were no significant differences between the study groups in the rates of serious clinical adverse events (10.3% with TDF–FTC and 10.9% with placebo, P=0.90) or laboratory adverse events (Table 2, and

Table 2. Adverse Events, According to Treatment Group.*

Adverse Event	TDF–FTC (N=611)		Placebo (N=608)		P Value†
	no. of participants (%)	*no. of events*	*no. of participants (%)*	*no. of events*	
Any	557 (91.2)	4357	536 (88.2)	4390	0.003
Any serious	63 (10.3)	68	66 (10.9)	79	0.90
Grade 3 or 4 only	19 (3.1)	21	29 (4.8)	32	0.17
At least possibly related to study drug	20 (3.3)	21	27 (4.4)	29	0.35
Upper respiratory tract infection	231 (37.8)	385	241 (39.6)	439	0.84
Headache	227 (37.2)	390	226 (37.2)	411	0.73
Dizziness	92 (15.1)	109	67 (11.0)	82	0.03
Abdominal pain	155 (25.4)	215	156 (25.7)	217	0.78
Nausea	113 (18.5)	132	43 (7.1)	48	<0.001
Vomiting	69 (11.3)	87	43 (7.1)	47	0.008
Diarrhea	76 (12.4)	93	65 (10.7)	76	0.22
≥5% Weight loss	75 (12.3)	113	61 (10.0)	72	0.13
Back pain	57 (9.3)	72	68 (11.2)	90	0.37
Rash	39 (6.4)	44	42 (6.9)	48	0.81
Fracture	7 (1.1)	7	6 (1.0)	8	0.74
Elevated creatinine	1 (0.2)	1	0	0	1.00
Hypophosphatemia	142 (23.2)	219	159 (26.2)	245	0.65
Hyperamylasemia	315 (51.6)	997	302 (49.7)	1017	0.45
Elevated AST	36 (5.9)	43	38 (6.2)	42	0.90
Elevated ALT	38 (6.2)	48	43 (7.1)	66	0.57
Death‡	2 (0.3)	2	4 (0.7)	4	0.45

* ALT denotes alanine aminotransferase, and AST aspartate aminotransferase.
† All P values were calculated with the use of a time-to-first-event analysis (regression analysis of survival data on the basis of the Cox proportional-hazards model), with the exception of the P values for weight loss of 5% or more and death, which were calculated with the use of Fisher's exact test.
‡ The causes of death in the TDF–FTC group were motor vehicle accident (one participant) and suicide (one); the causes of death in the placebo group were motor vehicle accident (two), homicide (one), and cerebrovascular accident (one).

Table S4 in the Supplementary Appendix). There were 107 pregnancies among 101 women during the study. Neither the rate of pregnancy nor the rate of fetal loss in early pregnancy differed significantly between the study groups (pregnancy: 17.1% with TDF–FTC and 19.1% with placebo, P=0.58; fetal loss: 7.1% and 6.9%, respectively; P=1.00).

EFFECTS ON BONE MINERAL DENSITY

Among 109 participants in the TDF–FTC group and 112 in the placebo group in whom bone mineral density was measured, there was a decline in T scores and z scores for bone mineral density at the forearm, hip, and lumbar spine in participants who received TDF–FTC, as compared with those who received placebo (P=0.004 for both T scores and z scores at the forearm and P<0.001 for both scores at the hip and lumbar spine) (Table 3, with sex-specific data in Tables S5 and S6 in the Supplementary Appendix). Seven participants in the TDF–FTC group and 6 in the placebo group had a bone fracture after initiating the study treatment (P=0.74) (Table 2, and Table S7 in the Supplementary Appendix).

EFFICACY

A total of 36 participants became infected with HIV — 10 in the TDF–FTC group and 26 in the placebo group — which was equivalent to a protective efficacy of TDF–FTC as compared with placebo of 61.7% (95% confidence interval [CI], 15.9 to 82.6; P=0.03). With the exclusion of 3 par-

Table 3. Bone Mineral Density Scores.*

Assessment	Forearm			Hip			Lumbar Spine		
	TDF–FTC (N=109)	Placebo (N=112)	P Value	TDF–FTC (N=109)	Placebo (N=112)	P Value	TDF–FTC (N=109)	Placebo (N=112)	P Value
T score			0.004			<0.001			<0.001
Enrollment	−0.75	−0.58		0.44	0.53		−0.72	−0.59	
6 mo	−0.77	−0.50		0.33	0.57		−0.84	−0.45	
12 mo	−0.79	−0.48		0.33	0.54		−0.77	−0.56	
18 mo	−0.93	−0.27		0.17	0.77		−0.92	−0.43	
24 mo	−0.92	−0.13		0.21	0.74		−1.11	−0.37	
z Score			0.004			<0.001			<0.001
Enrollment	−0.70	−0.54		0.45	0.54		−0.67	−0.54	
6 mo	−0.73	−0.45		0.35	0.58		−0.80	−0.41	
12 mo	−0.72	−0.42		0.34	0.55		−0.74	−0.53	
18 mo	−0.88	−0.21		0.18	0.78		−0.88	−0.41	
24 mo	−0.87	−0.13		0.20	0.76		−1.09	−0.28	

* In the TDF–FTC group, 58 participants completed bone mineral density testing at the 6-month visit, 45 at the 12-month visit, 36 at the 18-month visit, and 23 at the 24-month visit. In the placebo group, 66 participants completed bone mineral density testing at the 6-month visit, 44 at the 12-month visit, 33 at the 18-month visit, and 35 at the 24-month visit.

ticipants who were HIV-infected at the time of enrollment (1 in the TDF–FTC group and 2 in the placebo group), the overall protective efficacy of TDF–FTC in the modified intention-to-treat analysis (comprising 1216 participants) was 62.2% (95% CI, 21.5 to 83.4; P=0.03) (Fig. 2A). The incidence of HIV infection was estimated to be 1.2 cases per 100 person-years in the TDF–FTC group and 3.1 cases per 100 person-years in the placebo group. In the as-treated analysis, in which follow-up data for participants were censored 30 days after their last reported dose of study medication (with data censored for 4 participants in the TDF–FTC group and 19 in the placebo group), the protective efficacy was 77.9% (95% CI, 41.2 to 93.6; P=0.01) (Fig. 2B). TDF–FTC also had a protective effect in analyses of subgroups defined according to sex; however, the efficacy was not significant in all the analyses, owing to the occurrence of few end points in these subgroups (Table S8 in the Supplementary Appendix).

Virus from two HIV-infected participants showed antiretroviral resistance mutations (Table S9 in the Supplementary Appendix). In one participant in the TDF–FTC group, who had had unrecognized wild-type, acute HIV infection at the time of enrollment, K65R, M184V, and A62V reverse transcriptase resistance mutations developed at high levels (approximately 100%). After the diagnosis of HIV infection, the participant began receiving antiretroviral treatment with a combination of zidovudine, lamivudine, and lopinavir–ritonavir, with subsequent suppression of the plasma viral load to less than 400 copies per milliliter. In addition, one participant assigned to receive placebo had a K65R mutation intermittently and at very low levels (<1%) after seroconversion, although the mutation was not detected in the blood sample that had been obtained closest to the estimated date of seroconversion.

PLASMA DRUG LEVELS

Among the 4 participants in the TDF–FTC group who became infected with HIV during the study, 2 (50%) had detectable levels of tenofovir and emtricitabine in plasma obtained at the visit before and closest to their estimated seroconversion dates, whereas among the 69 participants, matched by sample date, who did not undergo seroconversion, 55 (80%) and 56 (81%) had detectable levels of tenofovir and emtricitabine, respectively (Fig. S8 in the Supplementary Appendix). The geometric mean detectable plasma concentrations of each drug were significantly lower among the participants who underwent seroconversion than among those who did not undergo seroconversion: 0.3 ng per milliliter (95% CI, 0.01, 8.02)

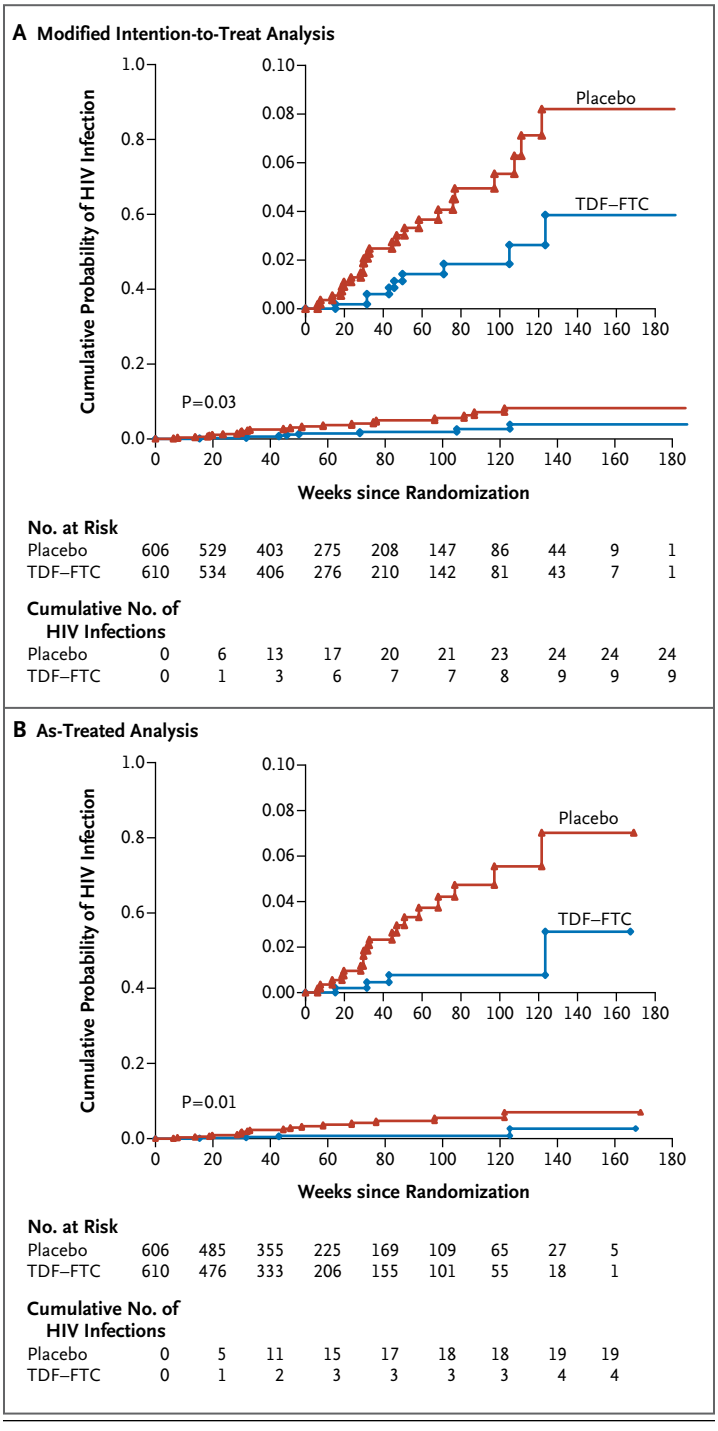

Figure 2. Kaplan–Meier Estimates of Time to HIV Infection.

Panel A shows the Kaplan–Meier estimates of the time to HIV infection in the modified intention-to-treat analysis, which included all study participants who were randomly assigned to receive a study medication, with the exception of participants who were retrospectively determined to have been infected with HIV at the time of enrollment. Panel B shows Kaplan–Meier estimates of the time to HIV infection in the as-treated analysis, in which follow-up data from participants in the modified intention-to-treat cohort were censored 30 days after participants reported receiving their last dose of study medication. The insets in both panels show the same data on an enlarged y axis.

vs. 30.6 ng per milliliter (95% CI, 16.3 to 57.5) for tenofovir (P=0.007) and 0.5 ng per milliliter (95% CI, 0.01 to 25.3) vs. 103.3 ng per milliliter (95% CI, 45.4 to 234.9) for emtricitabine (P=0.009). Neither drug was detected in any of the 19 participants in the placebo group who underwent seroconversion.

DISCUSSION

In this study of 1219 young, heterosexual adults in Botswana, TDF–FTC, taken orally once daily, decreased the rate of HIV infection by 62.2% when it was administered as part of a comprehensive package of HIV-prevention services. The protective efficacy was higher when the analysis was limited to participants who reported having taken the study medication within the previous 30 days, a finding that is consistent with increased efficacy among participants with high adherence to study medication in other trials of preexposure prophylaxis.[5,10,11] The rates of nausea, vomiting, and dizziness were higher among participants who were assigned to receive TDF–FTC than among those assigned to received placebo; these symptoms were transient and in most cases resolved rapidly. Over the course of 2 years of prophylaxis, we observed a small but significant decline in bone mineral density among participants taking TDF–FTC. We previously reported that 57% of our study population had low bone mineral density at the time of enrollment,[12] and our study population may therefore have been at increased risk for loss of bone mineral density while taking TDF. The clinical relevance of the observed decline in bone mineral density with respect to the risk of fracture remains uncertain. In other studies of HIV-negative persons receiving TDF as preexposure prophylaxis and in studies of previously untreated HIV-infected patients who were prescribed TDF–FTC as part of an antiretroviral therapy regimen, rates of fracture attributable to TDF exposure were not increased, despite a modest loss of bone mineral density; however, the duration of medication use and the length of follow-up were relatively short.[13,14]

We detected drug resistance among participants taking TDF–FTC who were infected with HIV at enrollment, an observation that has also been reported in other trials of preexposure prophylaxis.[4,15] The emergence of K65R, M184V, and A62V antiretroviral resistance mutations in a participant who had had unrecognized wild-type infection at the time of enrollment highlights the importance of careful HIV screening before and during preexposure prophylaxis. The low frequency of the K65R mutation detected intermittently in a participant in the placebo group falls within the reported natural polymorphism frequency for subtype C virus in the absence of drug,[16] suggesting that the mutation was probably not induced by tenofovir.

Our findings that participants who did not undergo seroconversion were more likely than those who did to have detectable plasma levels of drug and to have higher drug levels when detected highlight the critical importance of adherence. Both the Preexposure Prophylaxis Initiative (iPrEx) study and the Partners Preexposure Prophylaxis (Partners PrEP) study showed that the efficacy of preexposure prophylaxis depends largely on adherence to the medication, as assessed by measurement of plasma drug concentrations.[4,11] These findings are important to consider in light of the results of the Preexposure Prophylaxis Trial for HIV Prevention among African Women (FEM-PrEP) and the Vaginal and Oral Interventions to Control the Epidemic study (VOICE; ClinicalTrials.gov number, NCT00705679), both of which involved high-risk women.[7,8] Final analyses for the VOICE trial are pending, but the FEM-PrEP investigators noted that less than 40% of HIV-infected women and of HIV-uninfected women who were tested had detectable levels of study medication around the time of HIV infection and concluded that this poor adherence was likely to have contributed to the inability to identify efficacy.[17]

In our study, risky sexual behavior did not increase among the participants receiving study medication; however, taking a medication with known efficacy might lead to increased sexual disinhibition. Additional data from open-label and pilot implementation studies are needed to better understand the ways in which adherence to and acceptability of medication and potential increases in risky sexual behavior while taking medication alter the effectiveness of preexposure prophylaxis.

Our study has several limitations. First, the rates of study completion were lower than predicted, because more participants than expected withdrew from the study, mostly owing to relocation or conflicting obligations. Other researchers conducting studies in Botswana have noted difficulties in retaining this young, mobile, healthy population.[18] Our intensive efforts to reach participants who missed repeated visits ensured that 89.3% of all enrollees completed exit procedures and that final HIV infection status was known for 89.2%. Given the fact that the rates of study completion were similar in the two study groups, we believe that the lower-than-predicted retention rate did not confound or otherwise limit our findings. In addition, the total of 1563 person-years represented 80.2% of the person-years we had estimated we would need for our original power calculations. Second, our findings may not be generalizable to other populations, since we did not assess the efficacy of preexposure prophylaxis with TDF–FTC in preventing the transmission of HIV through anal sex or injection-drug use. Finally, we cannot state conclusively that TDF–FTC was protective for men and women independently, as was shown in the Partners PrEP study, which involved discordant couples who were prescribed TDF–FTC.[15]

In conclusion, daily oral TDF–FTC, given in the context of other prevention services, prevented HIV infection among heterosexual men and women. Additional data from other studies of the efficacy of preexposure prophylaxis and operational open-label research will help determine the effectiveness of programs that promote preexposure prophylaxis for the prevention of HIV infection.

The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the U.S. Centers for Disease Control and Prevention. Use of trade names is for identification purposes only and does not constitute endorsement by the Centers for Disease Control and Prevention or the Department of Health and Human Services.

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