ORIGINAL ARTICLE

Preexposure Chemoprophylaxis for HIV Prevention in Men Who Have Sex with Men

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ABSTRACT

BACKGROUND

Antiretroviral chemoprophylaxis before exposure is a promising approach for the prevention of human immunodeficiency virus (HIV) acquisition.

METHODS

We randomly assigned 2499 HIV-seronegative men or transgender women who have sex with men to receive a combination of two oral antiretroviral drugs, emtricitabine and tenofovir disoproxil fumarate (FTC–TDF), or placebo once daily. All subjects received HIV testing, risk-reduction counseling, condoms, and management of sexually transmitted infections.

RESULTS

The study subjects were followed for 3324 person-years (median, 1.2 years; maximum, 2.8 years). Of these subjects, 10 were found to have been infected with HIV at enrollment, and 100 became infected during follow-up (36 in the FTC–TDF group and 64 in the placebo group), indicating a 44% reduction in the incidence of HIV (95% confidence interval, 15 to 63; P=0.005). In the FTC–TDF group, the study drug was detected in 22 of 43 of seronegative subjects (51%) and in 3 of 34 HIV-infected subjects (9%) (P<0.001). Nausea was reported more frequently during the first 4 weeks in the FTC–TDF group than in the placebo group (P<0.001). The two groups had similar rates of serious adverse events (P=0.57).

CONCLUSIONS

Oral FTC–TDF provided protection against the acquisition of HIV infection among the subjects. Detectable blood levels strongly correlated with the prophylactic effect. (Funded by the National Institutes of Health and the Bill and Melinda Gates Foundation; ClinicalTrials.gov number, NCT00458393.)

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A TOTAL OF 2.7 MILLION NEW INFECTIONS with the human immunodeficiency virus (HIV) were diagnosed worldwide in 2008, according to the Joint United Nations Program on HIV/AIDS (UNAIDS). Combination antiretroviral therapy for patients with HIV infection restores health and may decrease the transmission of the virus to uninfected partners.[1] Therapy also decreases mother-to-child transmission.[2]

Postexposure chemoprophylaxis is recommended after occupational or nonoccupational exposure to HIV-infected fluids.[3] The use of such chemoprophylaxis requires that people recognize when they might have been exposed to HIV and that they start therapy within 72 hours. Both challenges are substantial limitations to the use of postexposure chemoprophylaxis.[4,5]

We selected emtricitabine (FTC) and tenofovir disoproxil fumarate (TDF) combination therapy in a single tablet (FTC–TDF) for evaluation of preexposure prophylaxis because of several favorable characteristics.[6] (Details are provided in the introduction in the Supplementary Appendix, available with the full text of this article at NEJM.org.) The protective activity of FTC and TDF has been shown in mice transplanted with human immune cells[7] and in nonhuman primates.[8-10] In these studies, there were increased levels of efficacy when both agents were used together, as compared with the use of either agent alone. The administration of the drug both before and after exposure was important for maximizing the protective benefit.[11]

Daily preexposure prophylaxis with oral TDF had an acceptable side-effect profile in a trial involving West African women.[12] A tenofovir 1% vaginal gel reduced HIV infection rates by 39% among women.[13] Men and transgender women who have sex with men are disproportionately affected by the global epidemic.[14,15] Surveys of such persons in the United States indicate that the current use of preexposure prophylaxis is rare, although the majority would consider such use if evidence of safety and efficacy became available.[16,17]

In this multinational study, called the Preexposure Prophylaxis Initiative (iPrEx) trial, we aimed to evaluate the safety and efficacy of once-daily oral FTC–TDF as compared with placebo for the prevention of HIV acquisition among men and transgender women who have sex with men.

METHODS

PROTOCOL DEVELOPMENT

We developed the concept and protocol for this study using methods that came to be approved as "good participatory practices" by UNAIDS.[18] The development of the protocol was sponsored by the National Institute of Health's Division of Acquired Immunodeficiency Syndrome (DAIDS). The protocol was approved by national government public health authorities in Peru, Ecuador, South Africa, Brazil, Thailand, and the United States and by the ethics committee at each site. All subjects provided written informed consent. The study coordinator vouches for the fidelity of the report to the protocol. The study protocol is available at NEJM.org, and a detailed description of the methods is provided in the Supplementary Appendix.

STUDY POPULATION AND RANDOMIZATION

Inclusion criteria were male sex at birth, an age of 18 years or older, HIV-seronegative status, and evidence of high risk for acquisition of HIV infection. Subject codes were randomly assigned in blocks of 10, stratified according to site. The subject codes were assigned consecutively at the study sites to eligible subjects at the time of the first dispensation of a study drug. Serologic testing for hepatitis B was performed at screening.

STUDY VISITS

Study visits were scheduled every 4 weeks after enrollment. Each 4-week visit included drug dispensation, pill count, adherence counseling, rapid testing for HIV antibodies, and taking of a medical history. Chemical and hematologic analyses were performed at weeks 4, 8, 12, 16, and 24 and every 12 weeks thereafter. During screening, a computer-assisted structured interview collected information about education level, self-identified sex, and alcohol use, along with subjects' perceived study-group assignment at week 12. High-risk behavior was assessed by interview every 12 weeks, and physical examinations and evaluations for sexually transmitted infections were performed at least every 24 weeks. Visits through May 1, 2010, are included in this report of the primary analysis of safety and efficacy. The visit cutoff date was set by the study sponsor without any access to interim findings and was intended

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to ensure observation of the targeted number of seroconversion events (85). The use of study drugs was intensively monitored and promoted (for details, see Methods in the Supplementary Appendix).

STANDARD PREVENTION INTERVENTIONS

At every scheduled visit, subjects received a comprehensive package of prevention services, including HIV testing, risk-reduction counseling, condoms, and diagnosis and treatment of symptomatic sexually transmitted infections, including gonorrhea and chlamydia urethritis, syphilis, and herpes simplex virus type 2 (HSV-2). In addition, at 24-week intervals, subjects were screened for asymptomatic urethritis, syphilis, antibodies to HSV-2, and genital warts and ulcers; treatment was provided when indicated. Sexual partners were offered treatment of sexually transmitted infections that were diagnosed in the subject. Subjects were linked to local prevention and treatment services when required to receive standard-of-care services. All subjects were instructed to protect themselves from HIV with conventional methods, since they were unaware of their study-group assignment. Subjects who reported a recent unprotected exposure to an HIV-infected partner were referred for postexposure prophylaxis (at sites where such therapy was available), and the administration of a study drug was temporarily suspended. Vaccination against hepatitis B virus (HBV) was offered to all susceptible subjects.

LABORATORY TESTING

Testing for HIV antibody was performed on whole blood with the use of two different rapid tests at every scheduled visit, and reactive rapid tests were tested with the use of Western blot analysis of serum (Fig. S1 in the Supplementary Appendix). Subjects with failed rapid tests were retested during the visit. HIV plasma RNA testing with the use of an assay with a lower limit of quantitation of 40 copies per milliliter was performed if seroconversion was detected within 12 weeks after enrollment. RNA testing was also used to identify the first date of laboratory evidence of infection for the as-treated analysis. Testing for drug-resistance genotyping and phenotyping was performed with the use of clinically validated assays on the basis of the viral load at the seroconversion visit.

SUBGROUP ANALYSIS OF DRUG LEVELS

A prespecified subgroup analysis was performed to investigate whether drug levels correlated with protective effect. Subjects with HIV infection were matched with two control subjects, one from each study group who were selected from among seronegative subjects, according to study site (Fig. S5 in the Supplementary Appendix). Plasma was tested for the presence of FTC and tenofovir (TFV), and peripheral-blood mononuclear cells were tested for FTC triphosphate (FTC-TP) and TFV diphosphate (TFV-DP), which are the active intracellular metabolites of FTC and TFV, respectively, with the use of validated liquid chromatography and tandem mass spectrometry assays.

STUDY OVERSIGHT

The study was designed by four of the investigators in collaboration with all the site investigators and communities. DAIDS reviewers approved the protocol, which was developed by the study investigators, and monitored the conduct of the trial at study sites. The Bill and Melinda Gates Foundation also provided funding but did not have a role in protocol development or site monitoring. Gilead Sciences donated both FTC–TDF and placebo tablets and provided travel-related support for meetings conducted by non-Gilead investigators. The role of Gilead Sciences in the development of the protocol was limited to sections regarding the handling of the study drugs. Neither Gilead Sciences nor any of its employees had a role in the accrual or analysis of the data or in the preparation of the manuscript. DAIDS agreed to give Gilead 30 days to comment on the manuscript, but there was no agreement to accept suggestions. The first author wrote the first draft of the manuscript (except for the drug-level sections, which were drafted by another investigator) and decided to submit the manuscript for publication. The protocol statistician and data manager vouch for the accuracy of the data, and the protocol chair and site investigators vouch for the completeness of the reported data.

STATISTICAL ANALYSIS

Data were collected on case-report forms and faxed to a DataFax server at DF/Net Research. It was determined that the observation of 85 incident HIV infections would yield a power of at least 80% with a one-sided alpha level of 0.05 to

reject a null hypothesis of efficacy of 30% or less if the true efficacy were 60% or more. The modified intention-to-treat analysis included available data for all subjects except those with HIV RNA detected in their enrollment sample. The as-treated analysis used a time-dependent covariate indication as to whether the subject was known to fall below the prespecified level of study-drug compliance (50%) on any of the following: records of study-drug dispensation alone, pill-use calculation on the basis of study-drug dispensation and returns, and subjects' self-report. For the as-treated analysis, pills from unreturned bottles were assumed to have been taken, and late visits were included in the analysis if the last dispensation allowed pill use on 50% or more of days. Safety analyses included all subjects.

Figure 1 (facing page). Enrollment and Outcomes.
The most common laboratory abnormalities that led to exclusion were elevations in hepatic aminotransferase levels, hyperbilirubinemia, and renal insufficiency. A total of 18 enrollees (0.7%) did not meet all eligibility criteria, including 2 subjects with preexisting diabetes mellitus, who were instructed to stop taking a study drug when the history was discovered. All enrolled subjects, including those who were subsequently found to be ineligible, were followed for HIV infection and safety. Quarterly-visit attendance is shown. Visits were considered to have been completed if they occurred before the subsequent visit window, with completion rates of 75 to 94% for all visits. The completion rate was more than 86% for all visits before week 132. Visits occurred within the protocol-defined window of ±5 days in 62 to 86% of visits.

RESULTS

STUDY SUBJECTS

Of 4905 subjects who were screened, 2499 were enrolled in the study from July 10, 2007, through December 17, 2009, at 11 sites in six countries (Fig. 1). The baseline characteristics of the two study groups were similar (Table 1). All subjects were born male, although 29 (1%) reported their current gender identity as female. The ages of the subjects ranged from 18 to 67 years; the FTC–TDF group was on average 9 months older than the placebo group (mean age, 27.5 vs. 26.8 years; P=0.04).

Among HBV-susceptible subjects at screening, 94% accepted HBV vaccination. We enrolled 13 subjects with chronic HBV infection that was detected at screening, and acute HBV infection was reported as an adverse event in 3 additional subjects (2 in the FTC–TDF group and 1 in the placebo group) after enrollment when elevated liver aminotransferase levels were observed. All the HBV infections resolved with detectable levels of immunity.

FOLLOW-UP AND ADHERENCE

The cohort was followed for 3324 person-years with a variable duration of observation (median, 1.2 years; maximum, 2.8 years) (Fig. 1). There were no significant trends in visit completion rates over time. Most subjects said they did not know their study-group assignment at week 12, and those who guessed their assignment were evenly distributed between the two groups (Table S3 in the Supplementary Appendix). No subjects were told their study-group assignment during the course of the trial. A study drug was temporarily discontinued in 21 subjects (8 in the FTC–TDF group and 13 in the placebo group) so that they could receive postexposure prophylaxis for HIV (P=0.28).

The rate of self-reported pill use was lower in the FTC–TDF group than in the placebo group at week 4 (mean, 89% vs. 92%; P<0.001) and at week 8 (mean, 93% vs. 94%; P=0.006) but was similar thereafter (mean, 95% in the two groups). At each visit, a portion of subjects (approximately 6%) did not report the number of pills missed. The percentage of pill bottles returned was 66% by 30 days and 86% by 60 days. The rate of pill use that was estimated according to pill count also increased during the first 8 weeks and then remained stable at a median ranging from 89 to 95%, depending on whether pills from unreturned bottles were counted as having been taken or not taken. On the basis of pill-dispensation dates and quantities, the rate of pill use decreased during the first year, from 99% to 91%, a trend that contrasted with pill counts and self-report, which indicated an increased rate of use.

SEXUAL PRACTICES

Sexual practices were similar in the two groups at all time points (P=0.97) (Fig. S2 in the Supplementary Appendix). The total numbers of sexual partners with whom the respondent had receptive anal intercourse decreased, and the percentage of those partners who used a condom increased after subjects enrolled in the study. There were no significant between-group differences in the numbers of subjects with syphilis (P=0.49), gon-

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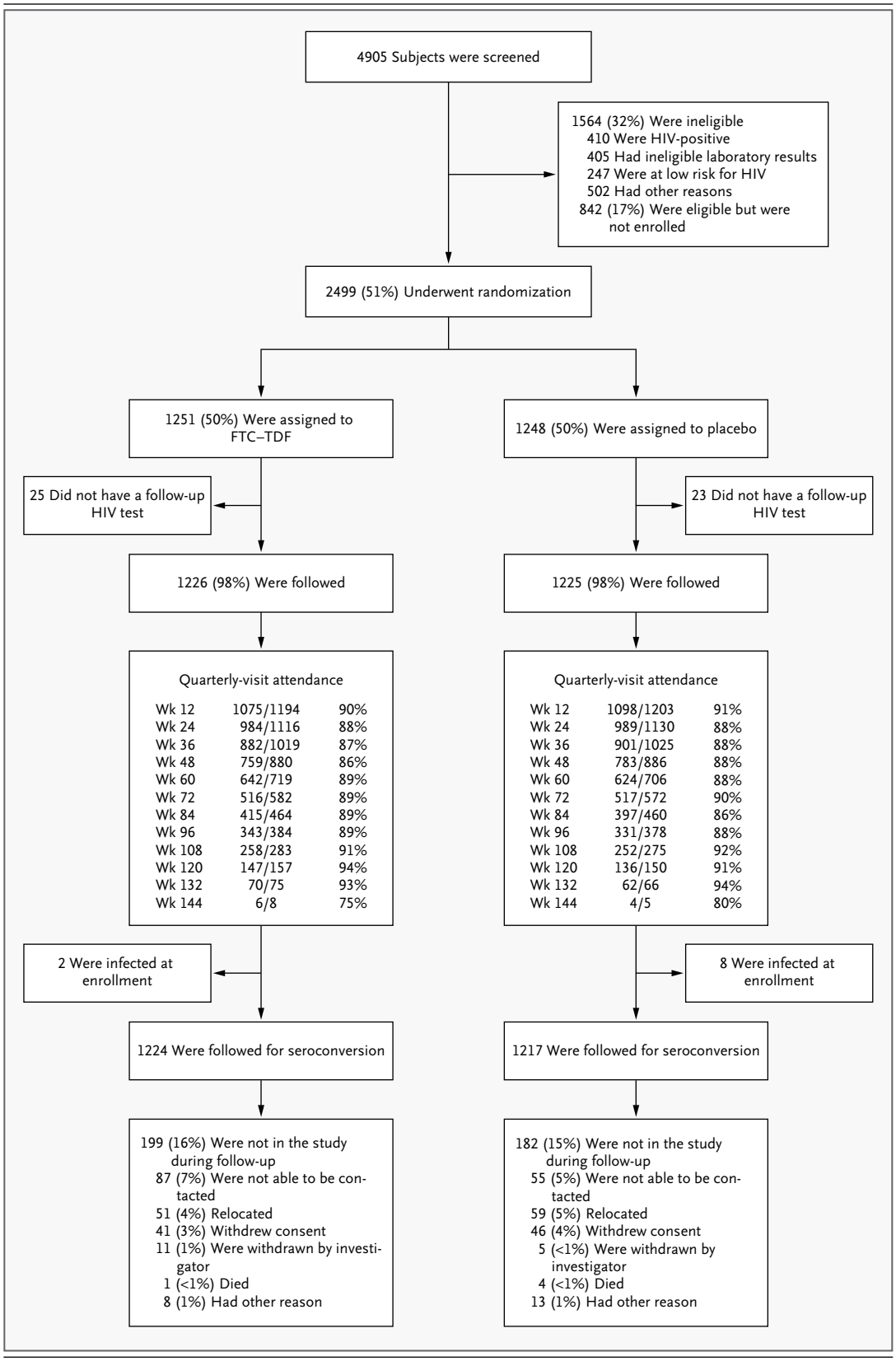

Table 1. Baseline Characteristics of the Subjects.*

Characteristic	FTC–TDF (N=1251)	Placebo (N=1248)	P Value
Age group — no. (%)			0.04
18–24 yr	591 (47)	662 (53)	
25–29 yr	274 (22)	241 (19)	
30–39 yr	249 (20)	224 (18)	
≥40 yr	137 (11)	121 (10)	
Education level — no. (%)			0.26
Less than secondary	279 (22)	244 (20)	
Completed secondary	430 (34)	453 (36)	
Postsecondary	525 (42)	539 (43)	
No answer or missing data	17 (1)	12 (1)	
Race or ethnic group — no. (%)†			0.40
Black	117 (9)	97 (8)	
White	223 (18)	208 (17)	
Mixed race or other	849 (68)	878 (70)	
Asian	62 (5)	65 (5)	
Hispanic	900 (72)	906 (73)	0.72
No. of alcoholic drinks (on days when subject drank in past month) — no. (%)			0.66
0	206 (16)	184 (15)	
1–4 per day	348 (28)	345 (28)	
≥5 per day	666 (53)	687 (55)	
No answer or missing data	31 (2)	32 (3)	
City and country of residence — no. (%)			1.00
Lima, Peru	470 (38)	470 (38)	
Iquitos, Peru	230 (18)	230 (18)	
Guayaquil, Ecuador	150 (12)	150 (12)	
Rio de Janeiro	147 (12)	147 (12)	
São Paulo	39 (3)	37 (3)	
San Francisco	70 (6)	70 (6)	
Boston	43 (3)	44 (4)	
Chiang Mai, Thailand	57 (5)	57 (5)	
Cape Town, South Africa	45 (4)	43 (3)	
Sexual risk factors at screening			
No. of partners in past 12 wk	18±35	18±43	0.51
Unprotected receptive anal intercourse in past 12 wk — no. (%)	732 (59)	753 (60)	0.37
Unprotected anal intercourse with partner with positive or unknown HIV status in past 6 mo — no. (%)	992 (79)	1009 (81)	0.34
Transactional sex in past 6 mo — no. (%)	517 (41)	510 (41)	0.84
Known partner with HIV in past 6 mo — no. (%)	23 (2)	32 (3)	0.22
Sexually transmitted infections diagnosed at screening			
Syphilis seroreactivity — no./total no. (%)	164/1240 (13)	162/1239 (13)	0.95
Serum herpes simplex virus type 2 — no./total no. (%)	458/1241 (37)	430/1243 (35)	0.24
Urine leukocyte esterase positive — no. (%)	23 (2)	22 (2)	1.00
Hepatitis B virus status — no. (%)			0.11
Susceptible	827 (66)	803 (64)	
Immune because of natural infection	247 (20)	222 (18)	
Immune because of previous vaccination	149 (12)	190 (15)	
Current infection with hepatitis B virus	7 (1)	6 (<1)	
Indeterminate	21 (2)	27 (2)	

* Plus–minus values are means ±SD. Percentages may not total 100 because of rounding. FTC–TDF denotes emtricitabine and tenofovir disoproxil fumarate.
† Race or ethnic group was self-reported.

Table 2. Adverse Events.*

Adverse Event	FTC–TDF (N=1251)		Placebo (N=1248)		P Value†
	no. of patients (%)	*no. of events*	*no. of patients (%)*	*no. of events*	
Any adverse event	867 (69)	2630	877 (70)	2611	0.50
Any serious adverse event	60 (5)	76	67 (5)	87	0.57
Any grade 3 or 4 event	151 (12)	248	164 (13)	285	0.51
Grade 3 event	110 (9)	197	117 (9)	225	0.65
Grade 4 event	41 (3)	51	47 (4)	60	0.57
Elevated creatinine level	25 (2)	28	14 (1)	15	0.08
Headache	56 (4)	66	41 (3)	55	0.10
Depression	43 (3)	46	62 (5)	63	0.07
Nausea	20 (2)	22	9 (<1)	10	0.04
Unintentional weight loss (≥5%)	27 (2)	34	14 (1)	19	0.04
Diarrhea	46 (4)	49	56 (4)	61	0.36
Bone fracture	15 (1)	16	11 (<1)	12	0.41
Death	1 (<1)‡	1	4 (<1)	4	0.18
Discontinuation of study drug					
Permanently	25 (2)	26	27 (2)	33	0.82
Permanently or temporarily	79 (6)	99	72 (6)	92	0.49

* A listing of all laboratory abnormalities and clinical adverse events of grade 2 or higher that were reported in 25 or more subjects (1%) is provided in Tables S9 and S10 in the Supplementary Appendix. FTC–TDF denotes emtricitabine and tenofovir disoproxil fumarate.
† P values were calculated by the log-rank test.
‡ This death was due to a motorcycle accident.

orrhea (P=0.74), chlamydia (P=0.43), genital warts (P=0.53), or genital ulcers (P=0.62) during follow-up (Table S4 in the Supplementary Appendix).

SAFETY

In testing for elevations in serum creatinine levels, there were 41 instances of elevations that were at least 1.1 times the upper limit of the normal range or more than 1.5 times the baseline level. Of these elevations, 26 (2%) were in the FTC–TDF group and 15 (1%) were in the placebo group (P=0.08). Two of these elevations increased in grade, accounting for a total of 43 creatinine adverse events (Table 2, and Table S9 in the Supplementary Appendix). Overall, 18 creatinine elevations (44%) remained in the normal range, and 36 (88%) were not confirmed on the next test. A total of 10 elevations led to discontinuation of a study drug (7 in the FTC–TDF group and 3 in the placebo group); study drugs were restarted in 9 subjects. Serum creatinine levels were elevated at more than one consecutive test in 5 subjects in the FTC–TDF group (<1%) and in none of the subjects in the placebo group. All elevations in the serum creatinine level resolved after the discontinuation of a study drug, within 4 weeks in 3 subjects, within 12 weeks in 1 subject, and within 20 weeks in 1 subject. Four of the subjects resumed taking FTC–TDF without recurrence of the elevation.

Moderate nausea (grade 2 and above) was reported more frequently in the FTC–TDF group than in the placebo group (22 vs. 10 events, P=0.04), as was unintentional weight loss of 5% or more (34 vs. 19 events, P=0.04) (for details, see Table S10 in the Supplementary Appendix).

EFFECT OF FTC–TDF ON HIV ACQUISITION

HIV rapid testing was performed at 39,613 visits, during which there were false reactive tests for 3 subjects at 7 visits; each subject had multiple negative tests afterward. HIV seroconversion was observed in 110 persons, of whom 10 had plasma HIV RNA subsequently detected in specimens obtained at the enrollment visit. A finding of fewer than 40 copies per milliliter of plasma HIV RNA was documented for the other 100 HIV-infected

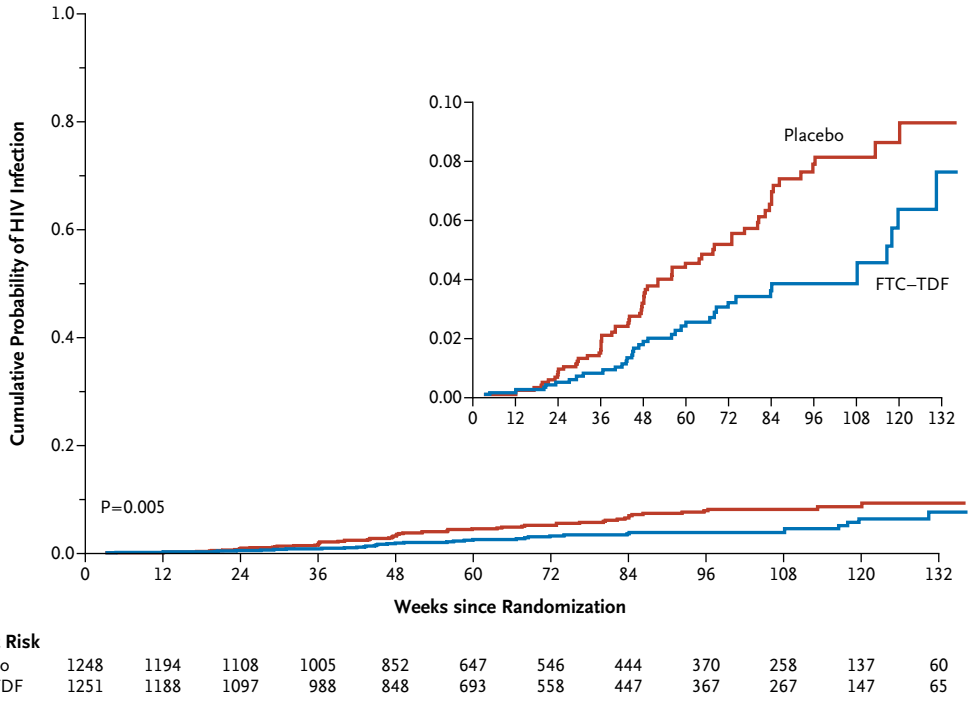

Figure 2. Kaplan–Meier Estimates of Time to HIV Infection (Modified Intention-to-Treat Population).
The cumulative probability of HIV acquisition is shown for the two study groups. The efficacy of preexposure prophylaxis with emtricitabine and tenofovir disoproxil fumarate (FTC–TDF) was 44%, as compared with placebo (P=0.005). The inset graph shows a more detailed version of the overall graph up to a probability of 0.10.

subjects before seroconversion. Among the 100 subjects with emergent HIV infection, 36 occurred in the FTC–TDF group, and 64 occurred in the placebo group, representing a relative reduction of 44% in incidence in the modified intention-to-treat population (95% confidence interval [CI], 15 to 63; P=0.005) (Fig. 2). After adjustment for the difference in age between the two groups, the efficacy was 43% (95% CI, 14 to 62). The rate of pill use on 50% or more of days was recorded on the basis of pill counts, self-report, and dispensation records at 81% of visits on which efficacy was 50% (95% CI, 18 to 70; P=0.006). This rate did not differ significantly (P=0.48) from the efficacy at visits with less than 50% pill use of 32% (95% CI, −41 to 67%) (Fig. 3). Efficacy of less than 30% could not be ruled out in the modified intention-to-treat analysis (P=0.15) or in the prespecified as-treated analysis at 50% pill use (P=0.09). There was no evidence of a change in HIV efficacy with longer follow-up (P=0.44).

In prespecified analyses of efficacy according to subgroup, efficacy was higher among subjects who reported at screening that they had previously had unprotected receptive anal intercourse than among those who did not (efficacy, 58%; 95% CI, 32 to 74) (Fig. 3). There was no significant between-group difference in protection on the basis of region, race or ethnic group, male circumcision, level of education, alcohol use, or age. In post hoc analyses, pill use on 90% or more of days was recorded at 49% of visits on which efficacy was 73% (95% CI, 41 to 88; P<0.001). Among all subjects, without exclusion for HIV infection at enrollment or the degree of compliance to the drug regimen, the efficacy was 47% (95% CI, 22 to 64; P=0.001).

Among the 10 subjects in whom plasma HIV RNA was subsequently detected in specimens obtained at enrollment, 5 had symptoms of an acute viral syndrome at enrollment, 2 had symptoms 1 week later (prompting an interim study visit), 1 had an anal sore, and 2 had leukopenia at enrollment. In these subjects, the clinicians did not suspect acute HIV infection, because the symptoms were attributed to an upper respira-

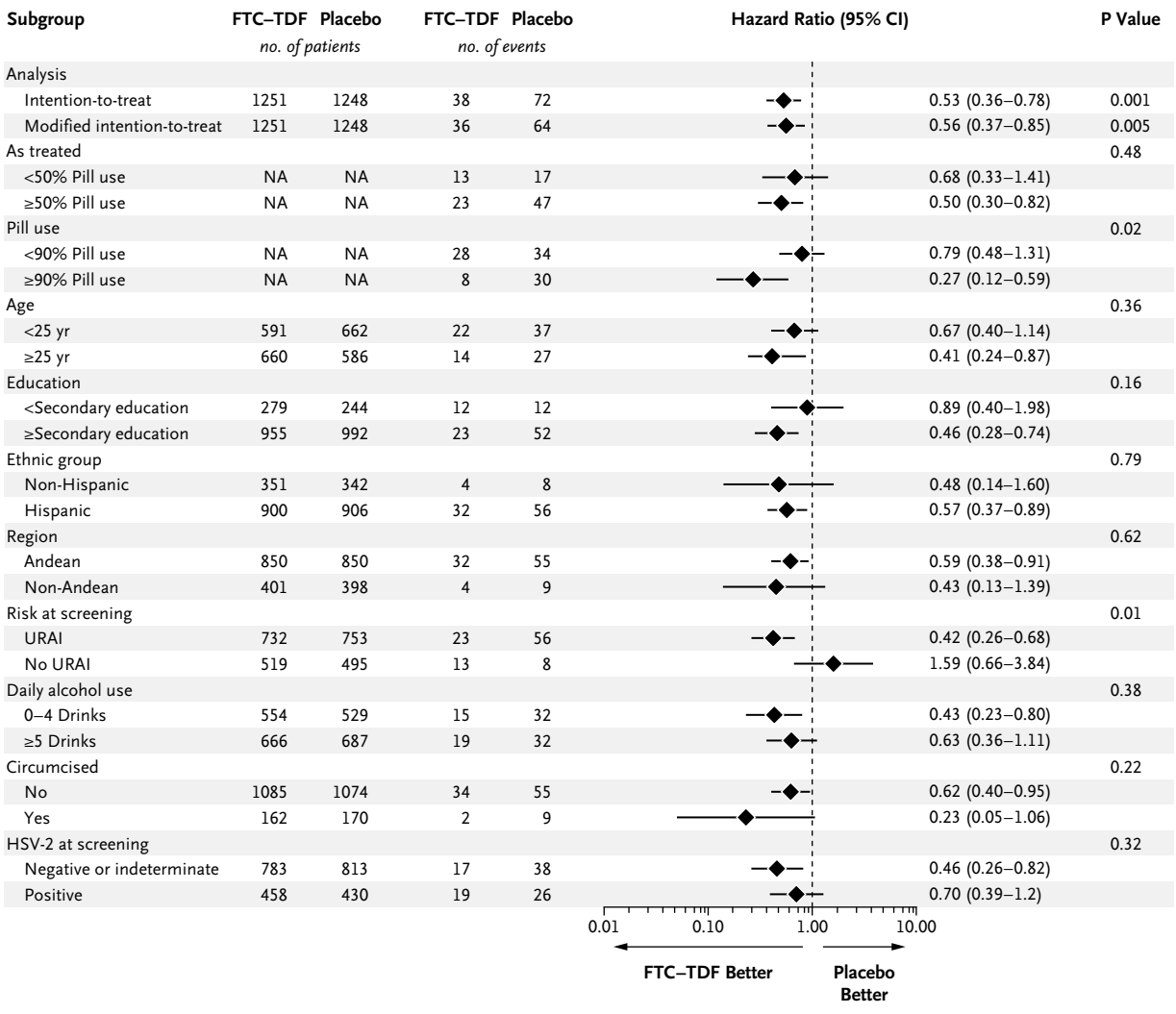

Figure 3. HIV Incidence among Subjects Receiving FTC–TDF, According to Subgroup.

The efficacy of emtricitabine and tenofovir disoproxil fumarate (FTC–TDF) is 1 minus the hazard ratio. Hazard ratios of less than 1 indicate efficacy, and 95% confidence intervals (shown by horizontal lines) that do not cross 1 indicate significant evidence of efficacy. All subgroup analyses were prespecified except for testing for herpes simplex virus type 2 (HSV-2) at screening and pill use at the rate of 90%. P values for the intention-to-treat analysis and the modified intention-to-treat analysis apply to the hypothesis of any evidence of efficacy; P values for other comparisons refer to the hypothesis that efficacy differed between the two strata. NA denotes not applicable, and URAI unprotected receptive anal intercourse.

tory tract infection, sinusitis, or other non-HIV cause.

Of the preexisting HIV infections at enrollment, two occurred in the FTC–TDF group and eight in the placebo group (P=0.06). Among subjects who were infected after enrollment, the numbers with detectable plasma HIV RNA before seroconversion were 5 of 36 (14%) in the FTC–TDF group and 7 of 64 (11%) in the placebo group (P=0.75). The time to seroconversion after RNA detection was similar in the two groups (P=0.55). After the discontinuation of a study drug, seroconversion rates were similar among 320 subjects (161 in the FTC–TDF group and 159 in the placebo group) (P=0.42). These subjects had a total of 1173 visits for HIV testing after the discontinuation of a study drug (642 in the FTC–TDF group and 531 in the placebo group).

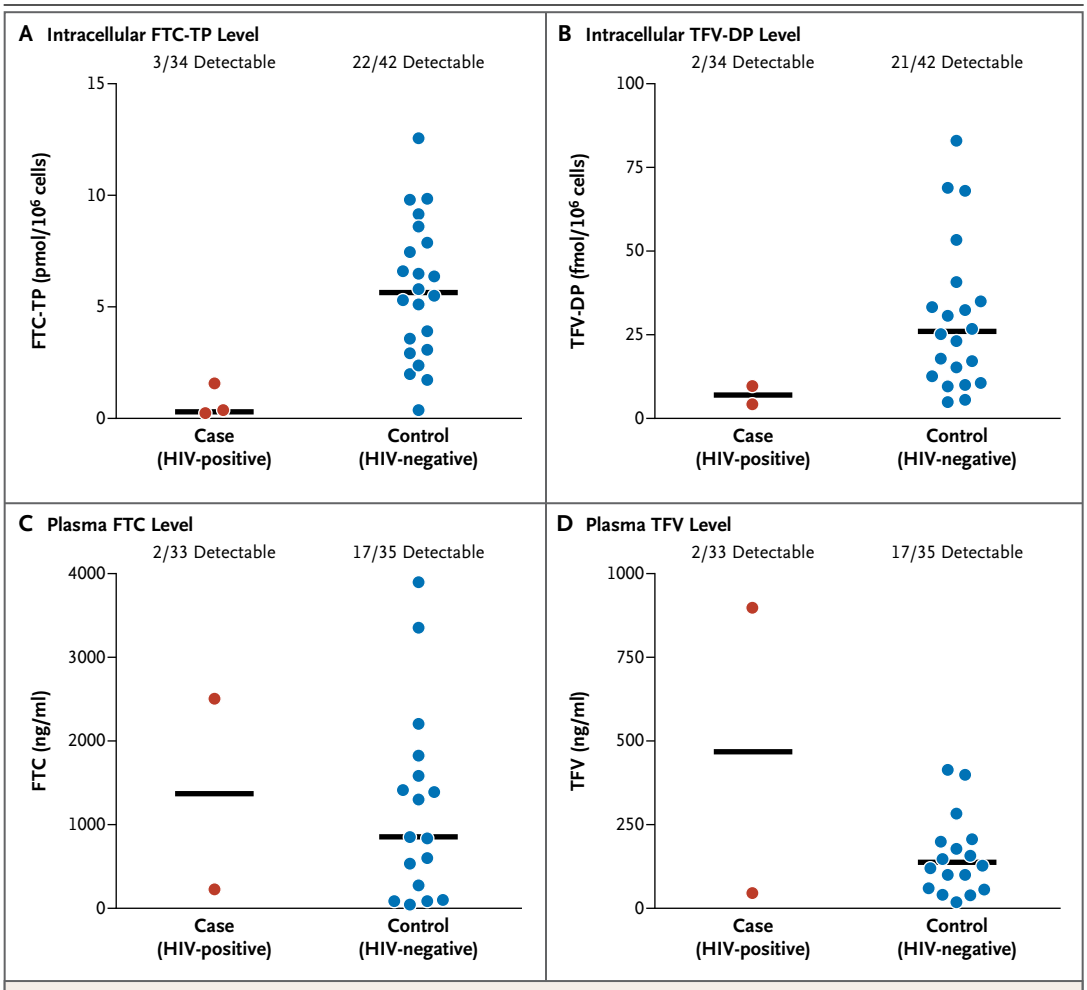

Figure 4. Levels of Study-Drug Components in Blood of Subjects Receiving FTC–TDF, According to HIV Status.
Shown are intracellular levels (Panels A and B) and plasma levels (Panels C and D) of components of emtricitabine and tenofovir disoproxil fumarate (FTC–TDF), quantified in specimens obtained from subjects in the FTC–TDF group. FTC-TP denotes emtricitabine triphosphate, and TFV-DP tenofovir diphosphate. The horizontal lines in each panel indicate medians.

During these visits, 5 seroconversions were observed (2 in the FTC–TDF group and 3 in the placebo group).

DRUG-LEVEL DETECTION AND PROPHYLACTIC EFFECT

Among subjects who became infected with HIV, the median time between the tested specimen date and the last uninfected visit was 35 days (interquartile range, 28 to 56). No drug was detected in any plasma or cell specimens from subjects in the placebo group. Among subjects in the FTC–TDF group, at least one of the study-drug components was detected in 3 of 34 subjects with HIV infection (9%) and in 22 of 43 seronegative control subjects (51%) (Fig. 4). Of the 3 HIV-infected subjects with a detectable level of a study drug, none had cell-associated drug levels higher than the median for the 22 seronegative control subjects in whom a study-drug component was detected. Only 8% of subjects with HIV infection and 54% of control subjects who were considered "on treatment" on more than 50% of days had a detectable level of a study drug in plasma or peripheral-blood mononuclear cells (Table S8 in the Supplementary Appendix). Detection of the different drug components was more than 95% concordant (Table S6 in the Supplementary Appendix).

In the FTC–TDF group, among subjects with a detectable study-drug level, as compared with

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those without a detectable level, the odds of HIV infection were lower by a factor of 12.9 (95% CI, 1.7 to 99.3; P<0.001), corresponding to a relative reduction in HIV risk of 92% (95% CI, 40 to 99; P<0.001). After adjustment for reported unprotected receptive anal intercourse, the relative risk reduction was 95% (95% CI, 70 to 99; P<0.001).

EFFECT OF FTC–TDF ON HIV INFECTION

Plasma HIV RNA levels and CD4+ T-cell counts were similar among subjects with seroconversion in the two groups (Fig. S4 in the Supplementary Appendix). Among the 10 subjects who were infected at enrollment, 3 had FTC-resistant infections (2 of 2 in the FTC–TDF group and 1 of 8 in the placebo group) (Table S5 in the Supplementary Appendix). No TDF-resistant infections were observed. Among 36 subjects in the FTC–TDF group and 64 subjects in the placebo group who became infected with HIV during the trial, no FTC or TDF resistance was detected.

DISCUSSION

Once-daily oral FTC–TDF provided 44% additional protection from HIV among men or transgender women who have sex with men who also received a comprehensive package of prevention services. The protective effect of FTC–TDF was significant but not as high as originally hypothesized during the design of the study. Although reported pill use was high, drug exposure that was measured objectively was substantially lower. The intracellular assay that was used in this study is expected to detect TFV-DP for 14 days or more after the last dose of TDF is taken (see Methods in the Supplementary Appendix). Other evidence of low drug exposure included the lack of drug resistance observed among emergent infections and the absence of suppression of the HIV RNA level in plasma at the seroconversion visit. There was no evidence of delayed seroconversion among subjects who were infected in the FTC–TDF group. More information will be available after the entire cohort stops receiving the study drug.

The estimate of biologic activity of FTC–TDF persists after adjustment for high-risk sexual practice, suggesting that the correlation between drug detection and protection is primarily due to the drug and not to other characteristics of subjects that may link poor adherence with higher risk. The testing of a larger number of specimens, from more subjects at more times, is needed to better define the minimum protective drug concentration. Protective drug levels may differ according to the type of exposure (rectal vs. penile). Drug level may have a role in monitoring trials, programs, and individual users. Methods for inexpensively measuring long-term drug exposure, such as that afforded by analysis of hair,[19] would be helpful once such a method is fully validated.

Side effects may have contributed to low pill use among some subjects. As with treatment of HIV infection and the use of FTC–TDF in postexposure prophylaxis,[20] the initiation of FTC–TDF preexposure prophylaxis was associated with self-limited start-up symptoms in a few subjects. The trial design involving a placebo may also have contributed to lower-than-expected pill use. All subjects were counseled that the study pill might be a placebo or an active drug having no proven benefit. Open-label research and program development could provide users with clearer information about expected benefits and risks, which might increase the use and efficacy of preexposure prophylaxis. Engagement with communities and additional behavioral research are needed to develop methods of counseling that better support such use.

The initiation of chemoprophylaxis either before or after exposure should be deferred in patients with signs or symptoms of a viral syndrome, which are often present during acute HIV infection.[21,22] The initiation of postexposure prophylaxis in patients who are RNA-positive but antibody-negative has been linked with acquisition of resistance to FTC and lamivudine (3TC),[5] as occurred in subjects in the FTC–TDF group who were already infected at enrollment in our trial. Ways to increase recognition of acute HIV infection would include routine measurement of body temperature and testing for HIV antibodies to evaluate viral syndromes, regardless of whether the presentation suggests HIV infection or another cause. Testing for HIV RNA at the time of the initiation of preexposure prophylaxis should be considered where available.

TDF treatment is known to cause decreases in renal function,[23] and there were trends toward more creatinine elevations in the FTC–TDF group than in the placebo group. Most creatinine elevations were self-limited and were not confirmed on repeat testing of a new specimen, as might occur due to dehydration, creatine use, or exercise. The ability to detect safety outcomes, including drug resistance, may have been decreased by

lower-than-expected drug exposure. In light of evidence of the efficacy of FTC–TDF, more information is needed about possible subclinical effects that may affect bone mineral density, low-level drug resistance, and proximal renal tubular function. Flares of hepatitis caused by HBV after stopping preexposure prophylaxis with TDF were not seen in West African women,[12] but more information is needed. These issues are being investigated in existing trials of preexposure prophylaxis.

Reported high-risk behavior decreased substantially after enrollment and remained lower than at baseline during the trial. Safer behavior was also observed in a trial of preexposure prophylaxis with TDF in West African women[12] and may reflect the services (e.g., counseling, testing, and dispensing of condoms) that are provided as part of such interventions. In addition, taking a pill a day may have served as a daily reminder of imminent risk and may have promoted planning for sex, which has been associated with lower HIV risk.[24] Behavioral changes during future open-label use of preexposure prophylaxis may differ because of an increased expectation of benefits, although such "risk compensation" was not observed during an open-label study of postexposure prophylaxis, during which benefits were expected.[25]

The optimal regimen for preexposure prophylaxis has not been established, and data from the subjects in our study cannot be applied to other populations. Alternative regimens in different populations are being studied. (Details are available in the Discussion in the Supplementary Appendix and at www.avac.org.)

In our study, preexposure prophylaxis with oral FTC–TDF among men and transgender women who have sex with men addressed an important unmet need in public health. HIV prevalence is higher in this population than in other groups in almost all countries.[14] In the United States, rates of HIV infection among such men and transgender women have climbed since the early 1990s, affecting in particular black and Hispanic subpopulations.[26] Intensive counseling in behavioral risk reduction for such subjects has not been shown to be better than standard counseling.[27] Although male circumcision partially protects heterosexual men,[28-30] penile circumcision is not expected to protect those who are exposed on the rectal mucosa.[31] Heterosexual women were partially protected by tenofovir 1% vaginal gel,[13] but the safety and utility of tenofovir topical gels for rectal use is not yet known. In the FTC–TDF group, there was increased efficacy among subjects who reported having unprotected receptive anal intercourse, which is the main mode of HIV transmission among the subjects in our study and increases the risk of heterosexual women who engage in the practice.[32] We showed that such subjects with a high risk of exposure to HIV can be mobilized to participate in prevention initiatives and that preexposure prophylaxis is effective for slowing the spread of HIV in this population.

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APPENDIX

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Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Supplemental Introduction

Few concepts for the prevention of sexual HIV transmission have been rigorously proven: of 37 late-phase trials, only 6 have demonstrated a significant protective benefit.[1,2] Tenofovir 1% vaginal gel had 39% efficacy in heterosexual women.[2] All other successful prevention interventions were clinic-based and directly observed, including enhanced services for sexually transmitted infections (STIs),[3] male circumcision,[4-6] and a vaccine candidate.[7] None of the successful interventions are known to be effective in men and transgender women who have sex with men (MSM), who carry a major burden of the global epidemic.[8,9]

Favorable characteristics of FTC/TDF for PrEP include the following: Both agents persist in active forms in the body for long periods of time, allowing for once daily dosing. Neither agent has interactions with anti-tuberculosis therapy, hormonal contraception, feminizing therapy, or anti-malarial agents. Both agents are used for treatment of HIV infection,[10] for which they have a well-established safety profile, and are available in patented and generic formulations.

Supplemental Methods

Protocol development

The trial was performed under US FDA IND #71,859 held by the NIH/NIAID/DAIDS. The study was initiated with protocol version 3 which included 4 sites in Peru and Ecuador. Version 4 of the protocol was implemented at all sites in June 2008, including new sites in Brazil, South Africa, Thailand and the United States when co-funding from the Bill and Melinda Gates Foundation became available. The decision to expand the study was made prior to enrolling the first participant and aimed to increase power and generalizeabilty. The study name iPrEx derives from the Spanish "Iniciativa Profilaxis Pre-exposicion" (PrEP initiative) and was selected by prospective participants.

Study populations

Screening visits to assess eligibility were to occur within 28 days of enrollment. Participants could rescreen one time. Screening procedures included informed consent, a computer assisted structured interview; HIV rapid testing and counseling; medical history and examination; screening for STIs using RPR, HSV-2 serum antibodies, and urine leukocytes esterase followed by GC/CT PCR if positive; HBV serologies (HBsAg, anti-HBc, anti-HBs, and anti-HBc IgM if anti-HBc was reactive); anti-HCV serological testing; and urine dipstick for glucose and protein. Enrollment procedures included informed consent, medical history, HIV rapid testing and counseling, and a blood draw for creatinine testing and specimen storage. The evidence of risk for acquisition of infection included any of the following in the 6 months prior to screening: anal sex with 4 or more male partners, a diagnosis of a sexually transmitted infection, history of transactional sex activity, or condomless anal sex with a partner who was HIV infected or of unknown infection status. Sites in Peru, Ecuador, São Paulo, and Boston required 6 or more partners, while sites in Chiang Mai, Cape Town, San Francisco and Rio de Janeiro required 4 or more partners. Other inclusion criteria were willingness to provide contact information and ambulatory performance ≥80 on the Karnofsky scale. Laboratory inclusion criteria changed between version 3 and version 4 of the protocol: version 3 required serum creatinine <= 1.2, ALT and AST < 2 times the upper limit of normal (ULN), total bilirubin <= 1.5 mg/dL, hemoglobin >10 g/dl, platelet count > 150,000 /mm3, an absolute neutrophil count greater than 1500 cells /mm3, and negative urine protein and glucose on urine dipstick at the

screening and enrollment visit. Version 4 of the protocol required serum creatinine <= ULN; AST, ALT, and total bilirubin <= 2 times ULN; hemoglobin >= 10 g/dl; platelet count within normal limits; an absolute neutrophil count of at least 1500 cells /mm3; and negative urine protein and glucose on urine dipstick in the 28 days prior to enrollment. Both versions required a creatinine clearance (estimated using Cockcroft-Gault) to be >= 60 mg/dl. Exclusion criteria were serious and active illness including diabetes requiring hypoglycemic agents, tuberculosis, and cancer requiring further therapy. Substance use sufficient to impair compliance with visits was excluded at the discretion of the site investigator. Use of nephrotoxic agents was excluded at enrollment (see Table S1). Persons reporting a history of pathological bone fracture not related to trauma were excluded. Also excluded were persons who had definitely or possibly received antiretroviral drugs or an anti-HIV vaccine while participating in a blinded clinical trial, or were concomitantly participating in a clinical trial or cohort study other than the iPrEx substudies. All participants provided written informed consent in their native language. Persons with chronic active hepatitis B infection (HBV) could be enrolled provided they were informed of their serological results and the special risks and benefits of FTC/TDF use, and consented to undergo 24 weeks of follow-up after stopping study drug. Participants with acute HBV infection, indicated by detection of anti- HBc IgM were excluded. Persons with active HBV infection were not enrolled at Brazilian sites. Non-literate participants received support from a participant advocate.

Visit Procedures

In addition to the procedures described in the main text, every 12-week visits also had a sexual behavior interview by interviewer and a computer assisted structured interview (CASI) and plasma and serum storage. Participants were allowed to plan to skip one visit every 24 week period. Study medication was dispensed as a single bottle of 30 tablets at enrollment. At follow-up visits, one un-empty and non-expired bottle could be redispensed after pill counting with a new bottle if required to cover the next visit interval. If a participant planned to skip a visit, he would receive 2 bottles. Replacement bottles were used for some participants whose pre-labeled drug inventory had become exhausted: these replacement bottles were coded in a manner that maintained the blind and were assigned by the drug manufacturer on a case-by-case basis. Persons who had reactive HIV rapid tests were followed every 2 weeks until their HIV-1 infection status was confirmed, and at week 4, 8, 12 after their positive rapid test and every 12 weeks thereafter. Physical exams were performed every 12 weeks in version 3 and every 24 weeks in version 4, and when warranted based on symptoms at all visits in both versions of the protocol. Sexually transmitted infection (STI) evaluation was performed when warranted by symptoms and every 24 weeks. Serum and plasma was stored at enrollment and every 12 weeks, and serum alone was stored at visits at weeks 4, 8, and 16. PBMCs were stored at enrollment and every 24 weeks, when drug was stopped, and at the seroconversion visit.

Monitoring and Promotion of Pill Use

Pill use was monitored by self-report during an interview and by clinic-based pill counts at visits when pills were either dispensed or suspended, and by comparing the number of pills dispensed at each visit with the time interval between visits (dispensation adherence). All participants were instructed to return all bottles at all visits. Estimates of pill use by pill count assumed that no pills were taken from unreturned bottles (lower estimate) or that all pills were taken from unreturned bottles (higher estimate). The higher estimate was used in the as treated analysis. At all on-treatment visits, participants received counseling encouraging daily pill use, including the importance of taking the pill every day. An interactive, client-centered, motivational interviewing based approach for study pill use was implemented for all participants starting between November

2009 and February 2010. Called "Next Step" counseling, the approach separates adherence assessment from counseling, to address social desirability bias in adherence reporting and to focus explicitly on barriers and facilitators of pill use, regardless of participants' reported level of use.[11]

Adverse Event Reporting

All adverse events were graded using the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, 2004 (DAIDS AE Table), except that grade 1 creatinine elevations could have a lower threshold. Grade 1 creatinine was defined according to the DAIDS AE Table or if the creatinine was 50% greater than baseline (defined as the average of screening and enrollment for the participant) or estimated creatinine clearance was <50 ml/min (calculated using Cockcroft-Gault). Adverse events were reported for Grade 2 and above clinical and laboratory abnormalities. In addition, all bone fractures and all creatinine elevations were reported. Serious adverse events (SAEs) were defined in accordance with the ICH, as any untoward medical occurrence that, at any dose, results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, or results in persistent or significant disability/incapacity.

Lab Methods

HIV antibody rapid tests used were Oraquick and Clearview in the United States and Bioline and Determine in other countries. All reactive rapid tests were confirmed using an FDA-cleared Western Blot (BioRad). Evaluation of sexually transmitted infections (STI) included urine leukocyte esterase (LE), an RPR with confirmatory testing, and HSV-2 serology (Focus) with an index value of ≥3.5 to define the positive range. Positive LE tests were followed up with nucleic acid testing for gonorrhea and chlamydia by PCR of urine specimens. Urine was tested at screening by dipstick for urine glucose and protein. Plasma HIV-1 RNA levels were measured by the RealTime PCR test (Abbott) or by the Amplicor HIV-1 Monitor Test (Roche). Drug resistance genotyping was performed using the Trugene Genotyping Kit (Siemens) and phenotyping was performed using the PhenoSense Assay (Monogram). CD4+ T cell counts were measured by flow cytometry. Other laboratory testing was performed by local laboratories which participate in external quality assurance from the College of American Pathologists as managed by the NIH SMILE program. Laboratory audits were conducted annually or more frequently.

Analytical Pharmacology

Drug assays were conducted at the University of Colorado. Approximately 5 million viable cells per sample were shipped and stored in liquid nitrogen vapor phase until processing. Cells were thawed and recounted with an automated hemocytometer (Countess, Invitrogen, Carlsbad, CA). Viability was recorded prior to lysing with cold 70% methanol in water. The cell extract was stored at -80°C until assaying. Plasma was shipped with the cells or on dry ice and was also stored at -80°C until assaying.

Plasma TFV and FTC concentrations were assayed with a simultaneous validated LC-MS-MS method.[12] FTC and TFV in plasma are stable at -80°C for at least 3 years.[12] The quantification range for both drugs was 10 to 1500 ng/mL. Intracellular TFV-DP and FTC-TP were assayed with a highly sensitive, simultaneous, validated LC-MS-MS procedure. The quantifiable range for TFV-DP was 2.5 to 2000 fmol/sample and that for FTC was 0.10 to 200 pmol/sample. Two million cells were typically extracted for the assays. Results were adjusted for cell viability and reported as fmol or pmol per million viable cells. Both assay methods have been reviewed by the DAIDS Clinical Pharmacology Quality Assurance Program (CPQA).

Stored viable cell samples such as were used in this study have been used successfully for the measurement of intracellular TFV-DP in previous studies. Liu et al measured TFV-DP in 59 stored viable cell samples from 12 placebo recipients and 47 TDF recipients where the cells were stored for an average (range) of 520 (240 to 836) days before processing.[13] This is a similar duration as in the present study, 373 (99 to 904) days, and the samples were processed in the same laboratory and with the same methodology. There was no downward trend observed between TFV-DP and days in storage up to 836 days (2.3 years). Plasma and hair were also measured in the Liu study at concurrent time points, and TFV-DP detection in viable cells was 96% concordant with TFV detection in plasma and 91% concordant with TFV detection in hair. Fletcher et al also used stored viable cells to measure TFV-DP in paired PBMC and lymph node lymphocytes samples in a small pilot study (n=7).[14] TFV-DP was detectable in all the samples using a 20-fold less sensitive assay compared with the present study.[15] The median intracellular concentration for TFV-DP from stored viable PBMCs from these two previous studies was approximately 20-40 fmol/10^6 cells for this sample type, lower than that observed in pharmacology studies among HIV-infected subjects where PBMC samples were processed and lysed immediately (70-90 fmol/10^6 cells).[13-16] The concentration data for TFV-DP in the present study should be compared with the concentration range of 20-40 fmol/10^6 cells previously identified for stored viable cells.[13 14] The lower limit of detection for the assay used in this study (2.5 fmol) is approximately 10-fold below this concentration range for TFV-DP. FTC-TP has not been measured previously in stored viable cells, to our knowledge.

The intracellular assay used in this study is expected to detect TFV-DP for 14 days or more after the last dose taken, assuming an initial concentration range of 20-40 fmol/10^6 cells and a half-life of 150h.[13,14,16,17] Similarly, FTC-TP is expected to be detectable for 7 days or more after the last dose taken, assuming an initial concentration range of 2 pmol/10^6 cells and a half-life of 39h.[18] Detectable plasma concentrations of FTC and TFV, with half-lives of 10 to 14 hours, would be expected to last for approximately 2-3 days after dosing.[19]

Statistical Methods

Optical character recognition of images of case report forms was followed by 2 rounds of entry checking. Discrepancies were resolved by the sites using source documents.

A multinational independent data safety and monitoring board (DSMB) met 3 times during the study on November 2007, November 2008, and November 2009. They reviewed enrollment, retention, and safety at the first 2 meetings; there was one review of efficacy at 60 events at the last meeting. Stopping boundaries for efficacy were based on flexible alpha spending approach[26] with an O'Brien Fleming[27] use function to preserve a 0.05 level test of at least 30% efficacy.

The primary outcome is time from enrollment to first laboratory evidence of infection (either a positive antibody test or detectable HIV-RNA) censoring HIV negative participants at their last negative antibody test prior to the visit cutoff of May 1, 2010. An endpoint monitoring committee, blinded to treatment assignment, reviewed all events, ensured completeness of testing, and determined the first laboratory evidence of HIV infection. The endpoint committee consisted of Robert Grant, Robert Hance, and Christopher Eden.

The cumulative probability of HIV was estimated by the method of Kaplan and Meier and two-sided tests for efficacy of 0% were based on the logrank test. The test of > 30% efficacy used a Wald test to rule out a hazard ratio of 0.70. Efficacy was defined as one minus the hazard ratio estimated from a Cox proportional hazards[28] model stratified by site with the Efron[29] correction for ties. Subgroup

analyses calculated p-values for effect modification based on a Wald test.

Additional criteria for the as treated analysis were that participants were considered to be in the lower stratum of pill use starting 3 days after study drug was held. Using pill use as a time-dependent dependent covariate allowed participants to return to the pill using subgroup after 84 days of pill use to ensure that HIV infections that occurred while the participant was in the lower stratum of pill use were not ascribed to the higher. A Cox model with term for treatment assignment, (time-dependent) pill use stratum and their interaction was fit. Such an analysis included all valid HIV tests. The pre-specified as-treated efficacy was determined by deriving the effect of treatment of FTC/TDF to placebo among HIV tests in an upper stratum defined by >= 50% adherence. Pre-specified subgroup analysis included region, URAI, ethanol use, ethnicity, race, and circumcision status.

Time to first onset of laboratory and clinical events was compared using a two-sided 0.05 level logrank test. The relative hazard of drug detection on HIV infection in the nested case control study was estimated by conditional logistic regression.[9] Mean values of CD4 and HIV-RNA are compared using linear mixed models[30] with an unstructured covariance matrix.

To evaluate the relationship between drug detection and HIV-infection, exact conditional logistic regression was used. Exact methods avoid having to make large-sample approximations for p-values and confidence intervals.[8] Logistic regression can be used to estimate the hazard ratio from a proportional hazards model in a case-control study with time-matched cases and controls.[9] The formula 100 x (1-OR), was used to estimate the relative reduction in hazard of HIV infection due to detectable drug levels. The presence of any quantifiable drug concentration in the specimen was considered evidence of “detectable” drug. The absence of any quantifiable drug was considered “undetectable” drug.

Specimens for the Pharmacology Analysis

The specimens used for the pharmacologic studies are shown in the figure below. Samples were available from plasma collected at quarterly visits and PBMCs from 6 monthly visits, and additional plasma and PBMCs that were drawn on the date of the first HIV rapid antibody positive test in HIV+ seroconverters. For HIV infected cases, plasma and peripheral blood mononuclear cell (PBMC) specimens were selected from the visit having the first laboratory evidence of HIV infection. HIV-negative controls were tested at the study week of seroconversion for their matched cases. The design was to match the sample at the seroconversion visit from all HIV+ seroconversion cases in both arms with a sample from one HIV- placebo participant and from another HIV- active arm participant. Matching was by study week of the seroconversion visit, and study site. Given that the HIV infection cases were most likely exposed to HIV by unprotected sexual intercourse, HIV exposure in controls was enriched by selecting at random from among participants reporting unprotected receptive anal intercourse (URAI) at the time of the specimen. If such specimens were not available, a control was selected at random. A maximum difference in study duration of 12 weeks was allowed in the matching.

Supplemental Figures and Tables

Figure S1. HIV Testing Algorithm During Follow-Up

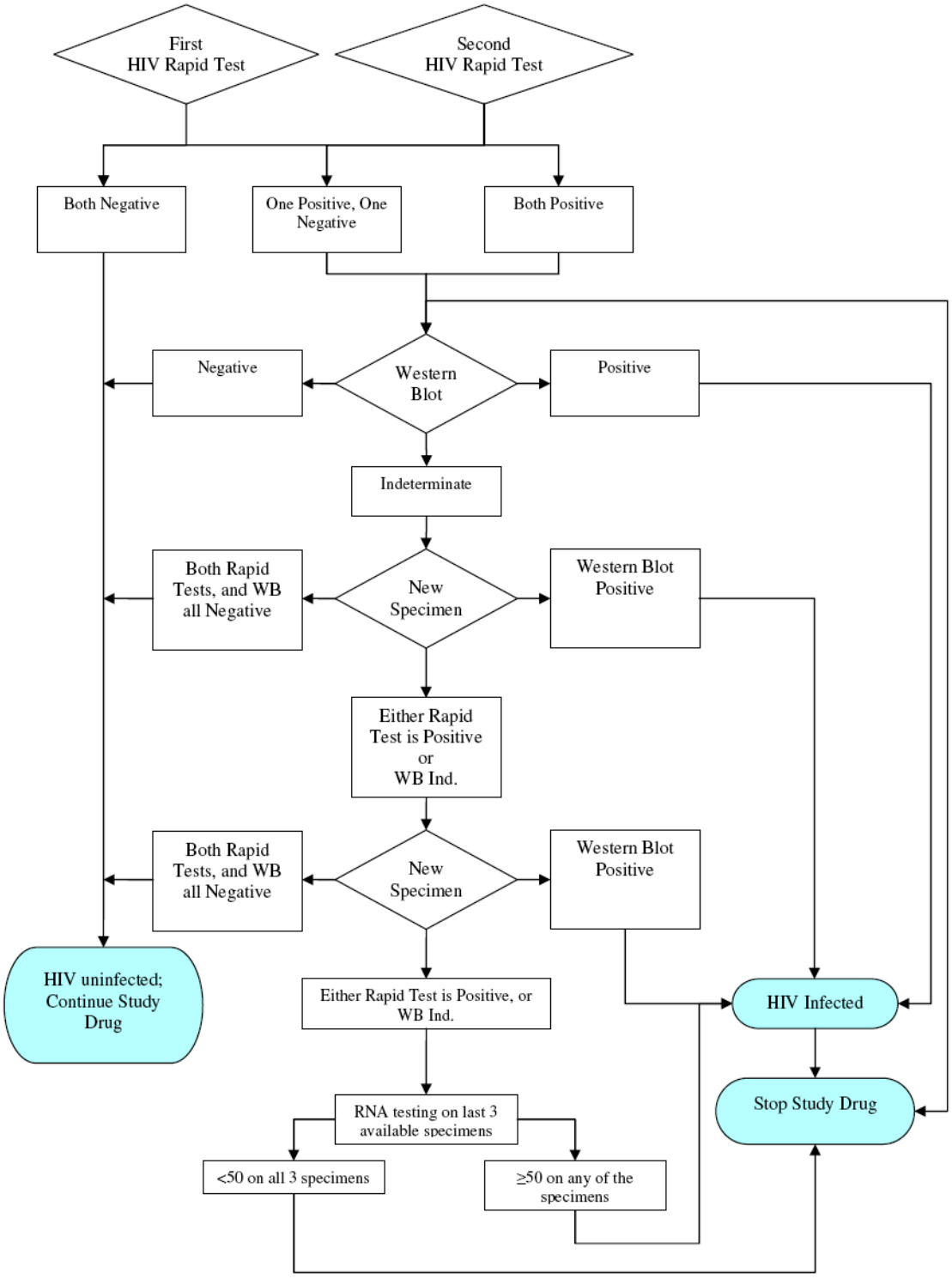


Table S1. List of Excluded Nephrotoxic Agents

Nephrotoxic Medications for iPrEx study

Please note that this list is not comprehensive. Clinicians should use their clinical judgment and consult with the iPrEx medical officer regarding other potentially nephrotoxic agents not listed here.

Excluded nephrotoxic agents

Aminoglycosides (amikacin, gentamycin, netilmycin, neomycin, streptomycin)
Amphotericin B
Acyclovir, intravenous
Adefovir
Bisphosphonates, intravenous (pamidronate, zoledronate)
Cidofovir
Carboplatin
Ceftazidime
Cisplatin
Clofarabine
Cyclophosphamide
Cyclosporine
Dextran (intravenous)
Flucytosine
Foscarnet
Gallium
Ganciclovir
Intravenous immune globulin (IVIG)
Lithium
Mannitol
Mesalamine
Mitomycin
Methotrexate
Oxaliplatin
Pemetrexed
Penicillamine
Pentamidine
Probenecid
Sirolimus
Sulfadiazine
Tacrolimus
Valganciclovir
Vancomycin

Acceptable agents

Acyclovir (oral)
Valacyclovir
ACE inhibitors
Thiazide diuretics (chlorthalidone, hydrochlorothiazide)
NSAIDS (require additional creatinine monitoring if taken for 7 consecutive days or more)

Version 1.0

Table S2. Hepatitis B Virus Infection Status at Screening in Enrolled Population

	FTC/TDF N=1251	Placebo N=1248
Hepatitis B (HBV) Status – no (%) P=0.11		
Susceptible (anti-Hbs neg, anti-HBc neg, HBsAg neg)	827 (66)	803 (64)
Immune due to Natural Infection (anti-HBs pos, anti-HBc pos)	247 (20)	222 (18)
Immune due to prior vaccination (anti-HBs pos, anti-HBc neg)	149 (12)	190 (15)
Current Hepatitis B Infection (HBsAg pos)	7 (1)	6 (0)
Indeterminate (anti-HBs neg, anti-HBc pos, HBsAg neg)	21 (2)	27 (2)

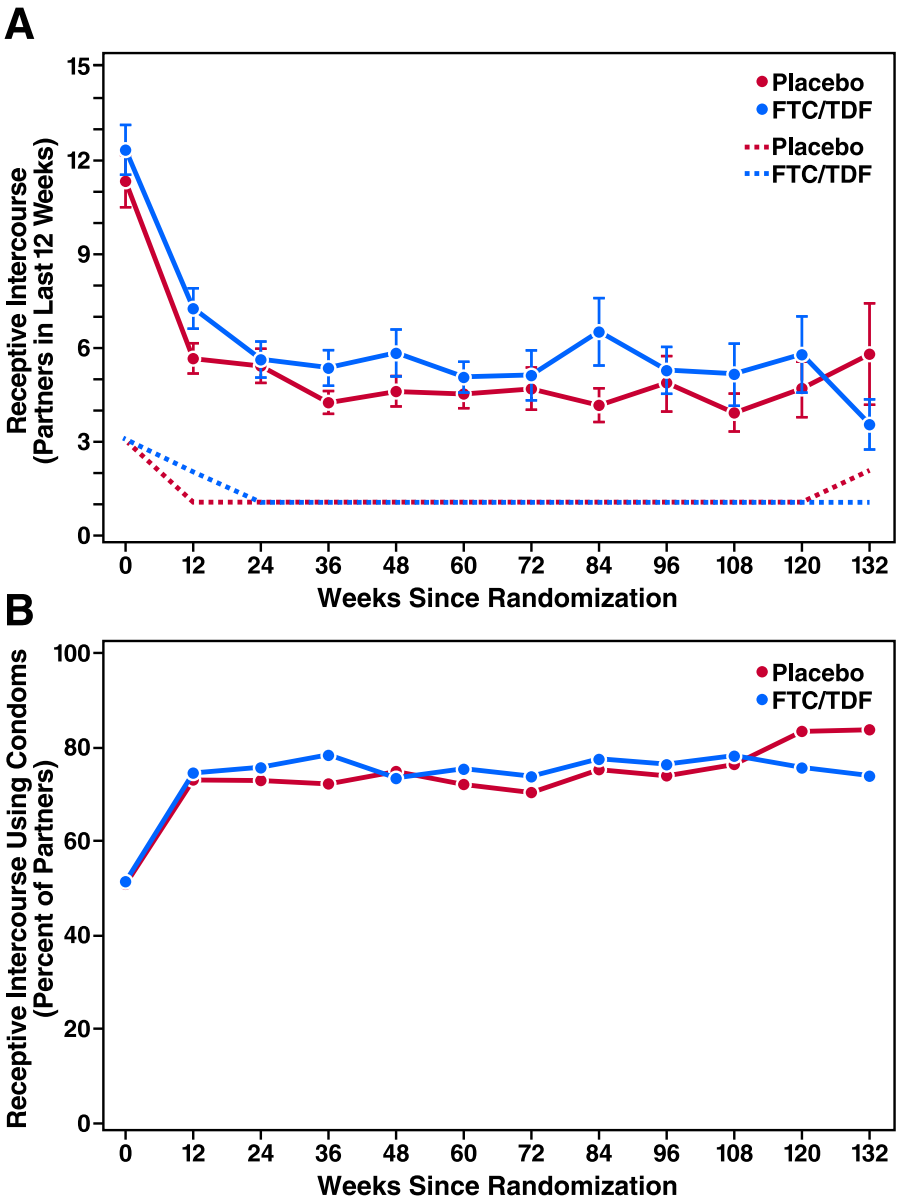
This information also appears in Table 1 of the article, and is reproduced here to specify the serological patterns used for each of the categories.

Table S3. Perceived Group Assignment At Week 12 By Randomized Group

Perceived Drug Assignment	Placebo	FTC/TDF	Overall
Strongly Truvada	131 (11%)	154 (13%)	285 (12%)
Somewhat Truvada	144 (12%)	124 (11%)	268 (11%)
Don't Know	719 (61%)	710 (61%)	1429 (61%)
Somewhat Placebo	86 (7%)	79 (7%)	165 (7%)
Strongly Placebo	29 (3%)	29 (3%)	58 (3%)
Decline to State	72 (6%)	74 (6%)	146 (6%)
Total	**1181 (100%)**	**1170 (100%)**	**2351 (100%)**

Perceived group assignment was recorded on a computer assisted structured interview at the week 12 visit. The majority of participants responded that the did not know their randomization group. The responses were evenly distributed by group (P=0.60 by Fisher exact test) indicating the integrity of the blinding.

Figure S2. Sexual Practices by Randomization Group.



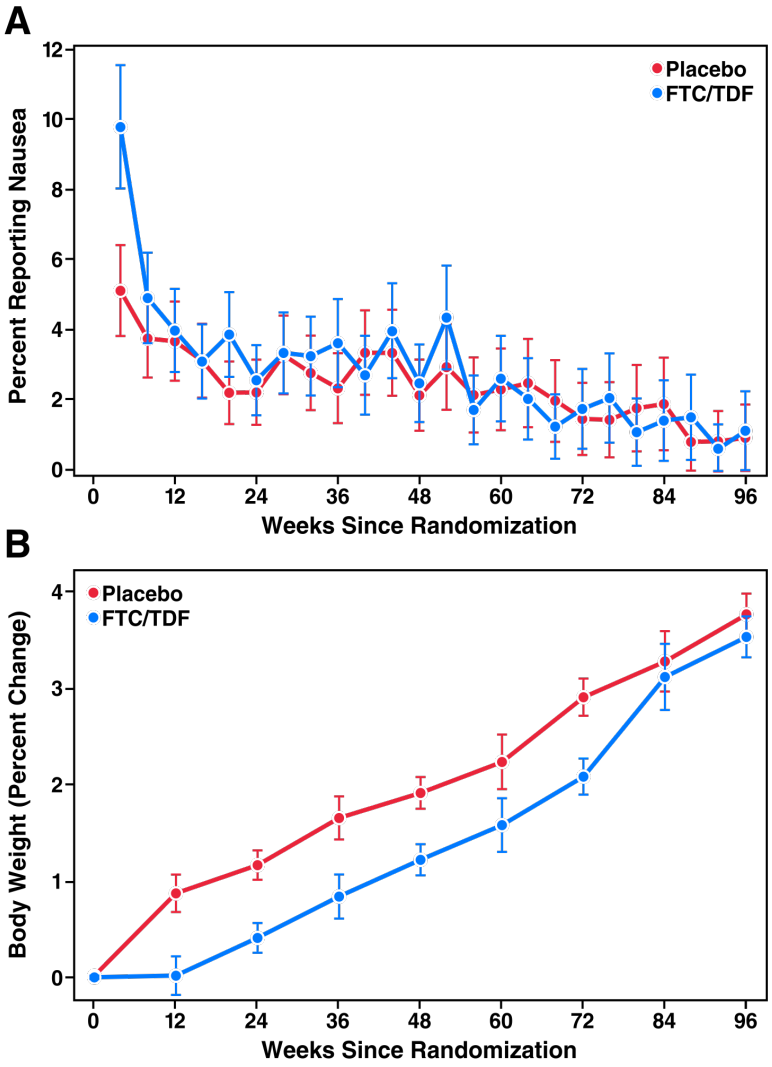
Partners with whom the participant had receptive anal sex in the previous 12 weeks (Panel A), and percentage of those partners using a condom (Panel B) by time on study and group. In Panel A, solid lines represent means and dotted lines represent median numbers, and the error bars are the standard error of the means.

Table S4. Participants with STIs by Visit and Randomization Group

Sexually Transmitted Infection	Study Rx	Study Week			
		24 N	48 N	72 N	96 N
Syphilis by RPR (P=0.49)					
	Placebo	165	145	111	70
	FTC/TDF	173	159	108	87
Warts by Exam (P=0.53)					
	Placebo	35	34	22	19
	FTC/TDF	44	37	26	15
Genital Ulcer by Exam (P=0.62)					
	Placebo	18	14	11	2
	FTC/TDF	18	11	6	2
Urethral Gonorrhea by PCR (P=0.74)					
	Placebo	8	6	2	1
	FTC/TDF	8	4	1	1
Urethral Chlamydia by PCR (P=0.43)					
	Placebo	8	2	3	1
	FTC/TDF	9	0	1	0

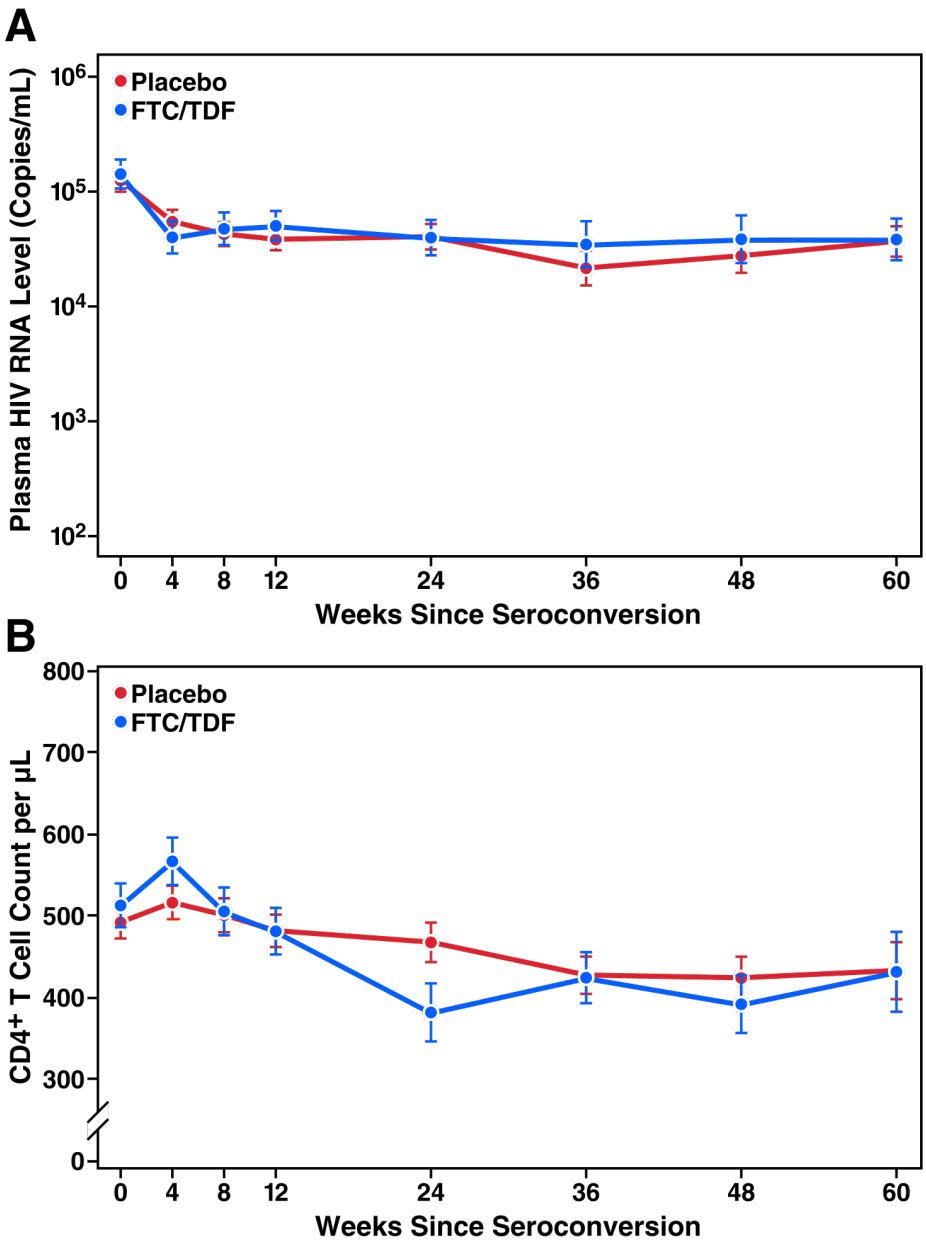
Medical examinations were performed at least every 24 weeks and laboratory testing for sexually transmitted infections (STIs) was performed every 24 weeks regardless of whether symptoms were reported. Gonorrhea and chlamydia PCR was performed if the urine leukocyte esterase was positive. P-values were calculated by the logrank test.

Figure S3. Nausea and Weight Change by Randomization Group



On monthly medical history questionnaires, nausea was more common during the first 4 weeks of pill use in the FTC/TDF group, occurring in 110 (9%) versus 58 (5%) in the placebo group (P<0.001), and then decreasing to lower and comparable levels in both groups at subsequent visits. The average weight increased at week 12 in the placebo group, but not the FTC/TDF group (0.9% vs 0%, P=0.002), and then increased about 1.5% per year in both groups. Overall weight loss of more than 5%, including both intentional and unintentional weight loss, was recorded for 15% in each group. Skin darkening was reported on monthly medical histories less frequently in the FTC/TDF group (8 versus 19 participants, P= 0.03).

Figure S4. Plasma RNA Level and CD4+ T Cell Count by Randomization Group



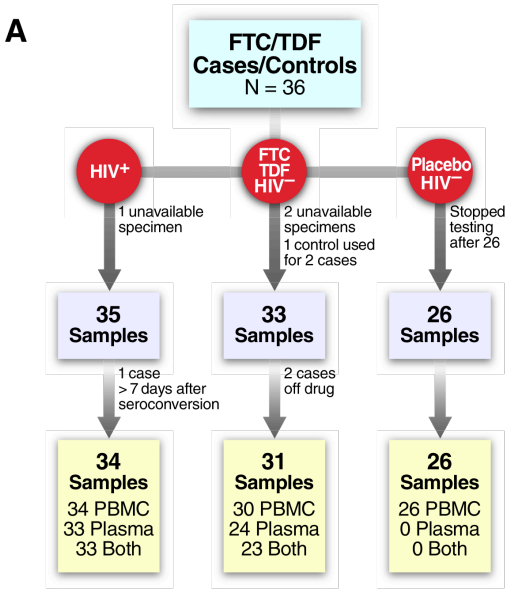
Plasma RNA level at the seroconversion visit was comparable in the two groups (5.15 versus 5.10 log10 copies/ml in the FTC/TDF and placebo groups respectively, P=0.72). At that visit, median reported pill use in the FTC/TDF group was 100% among respondents, of whom 25% reported pill use on less than 88% of days. An additional 21% said they did not know how many pills were missed and 5% were off treatment. Plasma RNA level was not lower in those reporting higher pill use or in the 3 seroconverters with detectable drug levels.

Table S5. Drug Resistance Findings

Case	Study Arm	Study Visit	Plasma HIV RNA Level (copies/ml)	Rapid Antibody Tests	Reverse Transcriptase Mutations Conferring Resistance	FTC Resistance Phenotype (Fold change FTC IC_{50})	Timing of Resistance
1	Placebo	Enrollment	417	Non-reactive	M184V, T215Y, and K103N	Not done	Primary
		W4	111,961	Reactive	M184V, T215Y, and K103N	>300	
2	FTC/TDF	Enrollment	10,000,000	Non-reactive	Wild type	Not done	Secondary
		W4	3,109*	Reactive	M184V	>300	
3	FTC/TDF	Enrollment	48	Non-reactive	Assay Failed	Not done	Indeterminate
		W4	<400*	Reactive	M184I	>300	

One case in the placebo group had primary or transmitted multidrug resistance to abacavir, didanosine, stavudine, zidovudine, FTC/3TC, nevirapine, and efavirenz conferred by 3 mutations (RT K103N, M184V, and T215Y). One case which occurred in the FTC/TDF group had FTC/3TC resistance conferred by the RT M184V mutation, and hypersusceptibility to zidovudine and TDF (fold change in IC50 of 0.36 and 0.46 respectively); there was no evidence of resistance at enrollment indicating that the drug resistance was acquired during the first 4 weeks of FTC/TDF use. The second case in the FTC/TDF group had FTC/3TC resistance conferred by the RT M184I mutation, with hypersusceptibility to zidovudine and TDF (fold change IC50 of 0.22 and 0.44 respectively); the enrollment plasma HIV RNA level was 48 copies/ml providing insufficient material for clinical resistance testing so the case may represent acquired or transmitted drug resistance. All together, 2 of 2 who enrolled with pre-existing infection and were assigned to the FTC/TDF group had FTC resistance at the week 4 visit, and 1 of 8 (12%, 95% CI 3-48%) in the placebo arm. *Tested for plasma HIV RNA level at week 8 after enrollment.

Figure S5. Specimens Used in The Nested Case Control Study of Drug Levels



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	Specimens	PBMC	Plasma	Both
FTC/TDF Cases:	34	34	33	33
FTC/TDF Controls:	43	42	35	34
Placebo Controls:	38	37	12	11

Forty-three active arm seronegative control specimens were available, 31 matched to FTC/TDF cases and 12 matched to placebo cases. Among the 36 active group cases, 29 had plasma and PBMC specimens available from the first seropositive visit, 2 had specimens from within 7 days after the visit, and 3 had specimens from a prior seronegative visit when HIV RNA was detected; all 34 were included in the analysis of HIV cases. Only HIV cases and their matched seronegative controls were used in the conditional logistic regression that compared the risk of HIV infection by drug detection the FTC/TDF arm.

Table S6. Concordance of Drug Detection in Plasma vs. Cells

(a)

TFV-DP	FTC-TP		
	Not Detected	**Detected**	Not Tested
Not Detected	51	2	0
Detected	0	23	0
Not Tested	0	0	1

(b)

TFV-DP	TFV		
	Not Detected	**Detected**	**Not Tested**
Not Detected	47	2	4
Detected	1	17	5
Not Tested	1	0	0

(c)

FTC-TP	FTC		
	Not Detected	**Detected**	**Not Tested**
Not Detected	47	0	4
Detected	1	19	5
Not Tested	1	0	0

(d)

TDF-DP	FTC		
	Not Detected	**Detected**	**Not Tested**
Not Detected	47	2	4
Detected	1	17	5
Not Tested	1	0	0

(e)

FTC	TFV		
	Not Detected	**Detected**	**Not Tested**
Not Detected	49	0	0
Detected	0	19	0
Not Tested	0	0	9

(f)

FTC-TP	TFV		
	Not Detected	**Detected**	**Not Tested**
Not Detected	47	0	4
Detected	1	19	5
Not Tested	1	0	0

Concordance of detectable or undetectable drug moieties among the active drug users was >95%.

Table S7. Case-Control Analysis of HIV Infection and Detectable Drug.

	Cases (HIV+) N=34		Active-Arm Matched Control (HIV-) N=43	
	Drug Detected N (%)	Drug NOT detected N (%)	Drug Detected N (%)	Drug NOT detected N (%)
ALL (N, %)	3 (9%)	31 (91%)	22 (51%)	21 (49%)
Reporting URAI	0 (0%)	13 (100%)	17 (47%)	19 (53%)
Reporting NO URAI	3 (14%)	18 (86%)	5 (71%)	2 (29%)

URAI refers to unprotected receptive anal intercourse. Detection of any drug moiety is stratified by any URAI was reported in 12 weeks prior to the specimen being tested.

Table S8. Comparison of Drug Detection by Adherence Strata

	Cases (HIV+) N=34		Controls (HIV-) N=43	
	Drug Detected	**Drug Not Detected**	**Drug Detected**	**Drug Not Detected**
"On drug" ≥50% Pill Use	**2/26 (8%)**	**24/26 (92%)***	**22/41 (54%)**	**19/41 (46%)**
"Off drug" <50% Pill Use	**1/7 (14%)****	**6/7 (86%)**	**0/2 (0%)**	**2/2 (100%)**

Expected concordant cells are shaded. Only 8% of cases and 54% of controls who were considered "on treatment" on more than 50% of days had detectable drug in plasma or PBMCs.

*one case had missing adherence information and undetectable drug.

**this case discontinued drug 7 days before the sampling visit as described in the text.

Table S9. Laboratory Abnormalities by Randomization Group

Laboratory Abnormality*	Study Rx	Maximum Grade				Number of	
		1	2	3	4	Participants	Events
Absolute Neutrophil Count: (p=0.76)							
	Placebo	24	2	1	1	28	35
	FTC/TDF	20	5	1	0	26	29
Total Hemoglobin (Low): (p=0.52)							
	Placebo	49	8	3	0	60	86
	FTC/TDF	42	9	3	0	54	78
Platelet Count (Low): (p=0.16)							
	Placebo	4	2	0	0	6	7
	FTC/TDF	7	3	2	0	12	14
Sodium (Low): (p=0.61)							
	Placebo	91	2	1	2	96	101
	FTC/TDF	99	1	1	1	102	113
Sodium (High): (p=0.61)							
	Placebo	214	5	1	0	220	276
	FTC/TDF	207	3	1	1	212	268
Potassium (Low): (p=0.70)							
	Placebo	32	0	0	0	32	34
	FTC/TDF	35	0	0	0	35	40
Potassium (High): (p=0.31)							
	Placebo	23	2	0	3	28	30
	FTC/TDF	33	3	0	0	36	40

*****This table includes laboratory abnormalities with onset date of May 1, 2010 or earlier. P-values are by the logrank test for the time to onset of the first laboratory abnormality. The numbers of participants are listed by the maximum grade they experienced. The number of events refers to the total number of events reported.

Table S9. Laboratory Abnormalities by Randomization Group (continued)

Laboratory Adverse Event	Study Rx	Maximum Grade				Number of	
		1	2	3	4	Participants	Events
Alkaline Phosphatase: (p=0.62)							
	Placebo	47	1	0	0	48	60
	FTC/TDF	50	3	0	0	53	73
ALT: (p=0.54)							
	Placebo	161	47	13	4	225	322
	FTC/TDF	149	47	12	4	212	292
AST: (p=0.40)							
	Placebo	147	31	13	3	194	251
	FTC/TDF	138	25	11	4	178	221
Total Bilirubin: (p=0.84)							
	Placebo	101	52	9	0	162	225
	FTC/TDF	115	38	4	1	158	230
Amylase: (p=0.85)							
	Placebo	74	16	5	1	96	120
	FTC/TDF	73	15	4	1	93	123

Table S9. Laboratory Abnormalities by Randomization Group (continued)

Laboratory Adverse Event	Study Rx	Maximum Grade				Number of	
		1	2	3	4	Participants	Events
Glucose (High): (p=0.20)							
	Placebo	232	41	3	0	276	367
	FTC/TDF	218	29	0	0	247	308
Creatinine: (p=0.08)							
	Placebo	12	1	1	0	14	15
	FTC/TDF	22	3	0	0	25	28
Phosphorus: (p=0.66)							
	Placebo	84	74	7	0	165	208
	FTC/TDF	74	86	11	0	171	225
C02/Bicarbonate: (p=0.47)							
	Placebo	106	1	0	0	107	132
	FTC/TDF	115	1	0	0	116	154
Leukocyte Count (Low): (p=0.32)							
	Placebo	5	1	0	0	6	6
	FTC/TDF	2	1	0	0	3	3

There were no differences between the groups in laboratory abnormalities related to liver function, amylase, electrolytes, glucose, phosphate, complete blood count, and absolute neutrophil count.

Table S10. Clinical Adverse Events by MedDRA Preferred Term and Group.

MedDRA Preferred Term*	Study Rx	Maximum Grade				Number of	
		1	2	3	4	Participants	Events
Abdominal Pain (P=0.14)							
	Placebo		13	2	0	15	15
	FTC/TDF	.	22	1	1	24	25
Abdominal Pain Upper (P=0.88)							
	Placebo	.	14	0	0	14	16
	FTC/TDF	.	13	0	0	13	19
Anogenital Warts (P=0.80)							
	Placebo	.	22	0	0	22	23
	FTC/TDF	.	20	0	0	20	23
Anxiety (P=0.32)							
	Placebo	.	24	2	0	26	31
	FTC/TDF	.	19	0	0	19	20
Arthralgia (P=0.83)							
	Placebo	.	13	2	0	15	17
	FTC/TDF	.	15	1	0	16	17
Back Pain (P=0.72)							
	Placebo	.	24	6	0	30	41
	FTC/TDF	.	26	1	0	27	35
Bronchitis (P=0.51)							
	Placebo	.	17	1	0	18	19
	FTC/TDF	.	11	3	0	14	18

*****This table includes adverse events with onset date of May 1, 2010 or earlier for MedDRA preferred terms which occurred in 25 (1%) or more of study participants. P-values are by the logrank test for the time to onset of the first adverse event. The numbers of participants are listed by the maximum grade they experienced. Grade 1 clinical adverse events were not reportable unless they were related to bone fracture. The number of events refers to the total number of events reported.

Table S10. Clinical Adverse Events by MedDRA Preferred Term and Group (continued)

MedDRA Preferred Term*	Study Rx	Maximum Grade				Number of	
		1	2	3	4	Participants	Events
Depression (P=0.07)							
	Placebo	.	50	5	7	62	63
	FTC/TDF	.	39	2	2	43	46
Diarrhea (P=0.36)							
	Placebo	.	54	2	0	56	61
	FTC/TDF	.	43	3	0	46	49
Flatulence (P=0.52)							
	Placebo	.	9	2	0	11	11
	FTC/TDF	.	14	0	0	14	14
Gastritis (P=0.47)							
	Placebo	.	20	5	0	25	29
	FTC/TDF	.	20	0	0	20	23
Gastroenteritis (P=0.59)							
	Placebo	.	24	2	0	26	31
	FTC/TDF	.	20	2	0	22	24
Gastrointestinal Infection (P=0.56)							
	Placebo	.	14	0	0	14	14
	FTC/TDF	.	16	1	0	17	19

Table S10. Clinical Adverse Events by MedDRA Preferred Term and Group (continued)

MedDRA Preferred Term*	Study Rx	Maximum Grade				Number of	
		1	2	3	4	Participants	Events
Genital Herpes (P=0.08)							
	Placebo	.	25	0	0	25	33
	FTC/TDF	.	14	0	0	14	30
Genital Ulceration (P=0.91)							
	Placebo	.	20	1	0	21	25
	FTC/TDF	.	20	0	0	20	23
Haematuria (P=0.59)							
	Placebo	.	24	1	0	25	25
	FTC/TDF	.	21	0	0	21	25
Headache (P=0.10)							
	Placebo	.	38	3	0	41	55
	FTC/TDF	.	54	2	0	56	66
Influenza (P=0.80)							
	Placebo	.	21	1	0	22	23
	FTC/TDF	.	17	3	0	20	22
Insomnia (P=0.97)							
	Placebo	.	14	0	0	14	14
	FTC/TDF	.	14	0	0	14	16

Table S10. Clinical Adverse Events by MedDRA Preferred Term and Group (continued)

MedDRA Preferred Term*	Study Rx	Maximum Grade				Number of	
		1	2	3	4	Participants	Events
Nasopharyngitis (P=0.55)							
	Placebo	.	24	2	0	26	28
	FTC/TDF	.	24	6	0	30	34
Nausea (P=0.04)							
	Placebo	.	9	0	0	9	10
	FTC/TDF	.	20	0	0	20	22
Pharyngitis (P=0.26)							
	Placebo	.	77	8	0	85	96
	FTC/TDF	.	61	9	0	70	85
Secondary Syphilis (P=0.64)							
	Placebo	.	25	0	0	25	25
	FTC/TDF	.	23	5	0	28	29
Sinusitis (P=0.75)							
	Placebo	.	17	0	0	17	17
	FTC/TDF	.	15	0	0	15	18
Syphilis (P=0.60)							
	Placebo	.	45	0	0	45	51
	FTC/TDF	.	49	0	0	49	59
Tinea Cruris (P=0.26)							
	Placebo	.	12	1	0	13	14
	FTC/TDF	.	18	1	0	19	19

Table S10. Clinical Adverse Events by MedDRA Preferred Term and Group (continued)

MedDRA Preferred Term*	Study Rx	Maximum Grade				Number of	
		1	2	3	4	Participants	Events
Tonsillitis (P=0.98)							
	Placebo	.	12	2	0	14	16
	FTC/TDF	.	14	0	0	14	15
Upper Respiratory Tract Infection (P=0.65)							
	Placebo	.	47	0	0	47	56
	FTC/TDF	.	42	0	0	42	53
Urethritis (P=0.21)							
	Placebo	.	63	0	0	63	71
	FTC/TDF	.	49	0	0	49	59
Weight Decreased (P=0.04)							
	Placebo	.	10	4	0	14	19
	FTC/TDF	.	22	5	0	27	34

Supplemental Discussion

The most likely explanation for the high rate of undetectable drug in this study was low pill use. Poor drug absorption or rapid clearance are unlikely given that FTC and TDF plasma pharmacokinetics have been studied in diverse populations including HIV-negative volunteers and Hispanics, who made up much of the iPrEx study sample, and no unusual patterns of undetectable drug have been reported.[19-21] The high concordance among positive and negative drug detection in plasma and cells and between FTC and TFV is also evidence against slow drug absorption or rapid clearance as causes for low drug levels. The intracellular assay used in this study was sensitive enough to detect drug for approximately 14 days after the last dose taken, assuming expected concentrations in stored viable specimens, which were used in this study, and the half-lives of 39h and 150h for FTC-TP and TFV-DP, respectively.[16,18] The detection of intracellular TFV-DP in stored viable cell specimens has been compared against TFV detection in other sample types, such as plasma and hair, with >90% concordance.[13]

High reported adherence with low objective indicators of use have been reported in heterosexual women in a microbicide trial,[22] as in this trial of MSM. Social desirability reporting bias may be higher in efficacy trials, which place a strong emphasis on perfect compliance: Strategies to allow comfort in accurate reporting are clearly needed.

Start-up symptoms could have contributed to drug interruptions that were not reported by the participants. Long-term adherence could be improved if peers or counselors provide reassurance that side effects will resolve after a few weeks.

Fewer participants in the FTC/TDF group were subsequently found to have pre-existing HIV infection at enrollment. FTC/TDF may have provided some post-exposure prophylactic benefit after enrollment. There was no evidence for delayed seroconversion in the FTC/TDF group in this trial. Occult infection and delayed seroconversion were not observed in non-human primates protected by PrEP regimens.[23] Additional information about possible post-treatment manifestations of PrEP use will be available after all iPrEx participants stop study drug.

The optimum PrEP regimen has not been established. Non-human primate models suggest that combination FTC/TDF is more protective than TDF alone, although adding FTC to the regimen was associated with drug resistance while TDF alone was not.[24] Clinical trials that include arms for both FTC/TDF and TDF alone are in progress (see www.avac.org). While the iPrEx study recommended once daily pill use to all participants, the levels of drug associated with protection could be achieved with less frequent dosing. Peri-intercourse use of a tenofovir 1% vaginal gel was efficacious for women.[2] Whether peri-intercourse dosing of oral FTC/TDF is acceptable, feasible and effective in MSM warrants further study, as this approach would decrease pill requirements and costs and may decrease dose-related side effects.

This study of FTC/TDF PrEP in MSM is not generalizeable to other populations, like heterosexual men and women, and injection drug users who are being evaluated in other PrEP studies. These populations have different routes of exposure to HIV (penile, vaginal, and parenteral), special safety concerns related to pregnancy, and social circumstances that may make pill use easier or more difficult. FTC/TDF PrEP was more effective in those reporting unprotected rectal exposure at baseline in this study; more information about PrEP efficacy after penile exposure is needed, and trials in heterosexual men are underway in Africa (see www.avac.org).

PrEP is a behavioral intervention requiring that services be available and used. Both are well-known challenges in the prevention field. Cost-effectiveness is important, and is favored by efficacy in high-risk groups, minimal monitoring requirements to assure safety, rare adverse events, and activity in younger populations.[25] The iPrEx study found greater efficacy in those reporting URAI at screening, the subgroup with the highest HIV incidence in the placebo arm. Finding safety and efficacy in young adult MSM, who comprised half of the iPrEx cohort, highlights important opportunities to protect people while social and behavioral skills are learned. Daily oral FTC/TDF PrEP was not associated with moderate or severe adverse events, confirming previous reports.[26]

Future research and program development should continue to build synergies between PrEP and other prevention strategies, including HIV testing and counseling, planning for sex, STI management, and HBV vaccination. Such mutually reinforced frameworks are needed to protect diverse communities from the spread of HIV and other diseases.

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