# 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. Alcohol use in Table 1 and Figure 3 was reported by CASI as the number of drinks per day on days in the last month when the participant drank alcohol. 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[20] with an O'Brien Fleming[21] 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[22] model stratified by site with the Efron[23] 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.[24] Mean values of CD4 and HIV-RNA are compared using linear mixed models[25] 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.[26] 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.[24] 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***

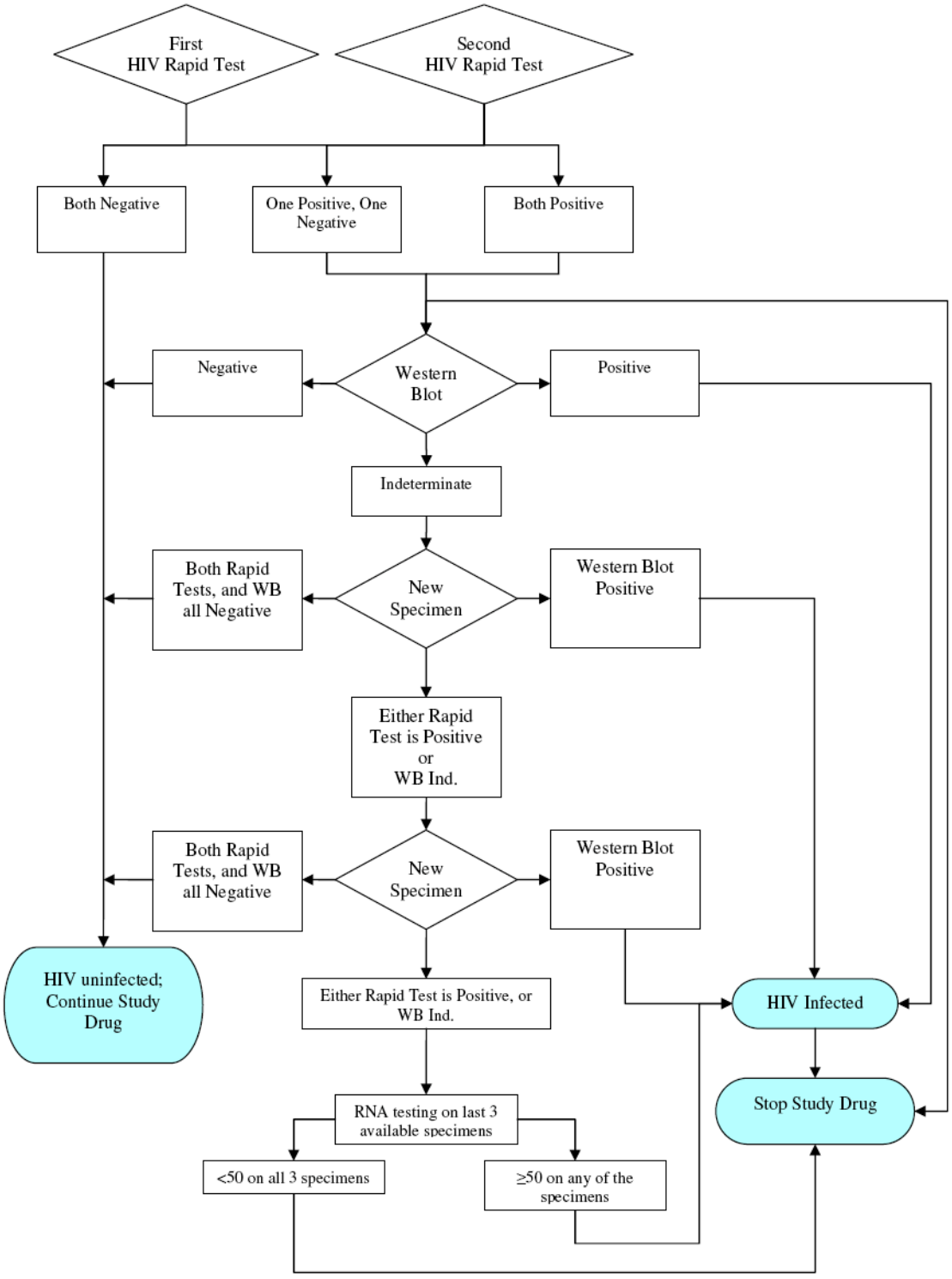


***Table S1. List of Excluded Nephrotoxic Agents***

**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) |

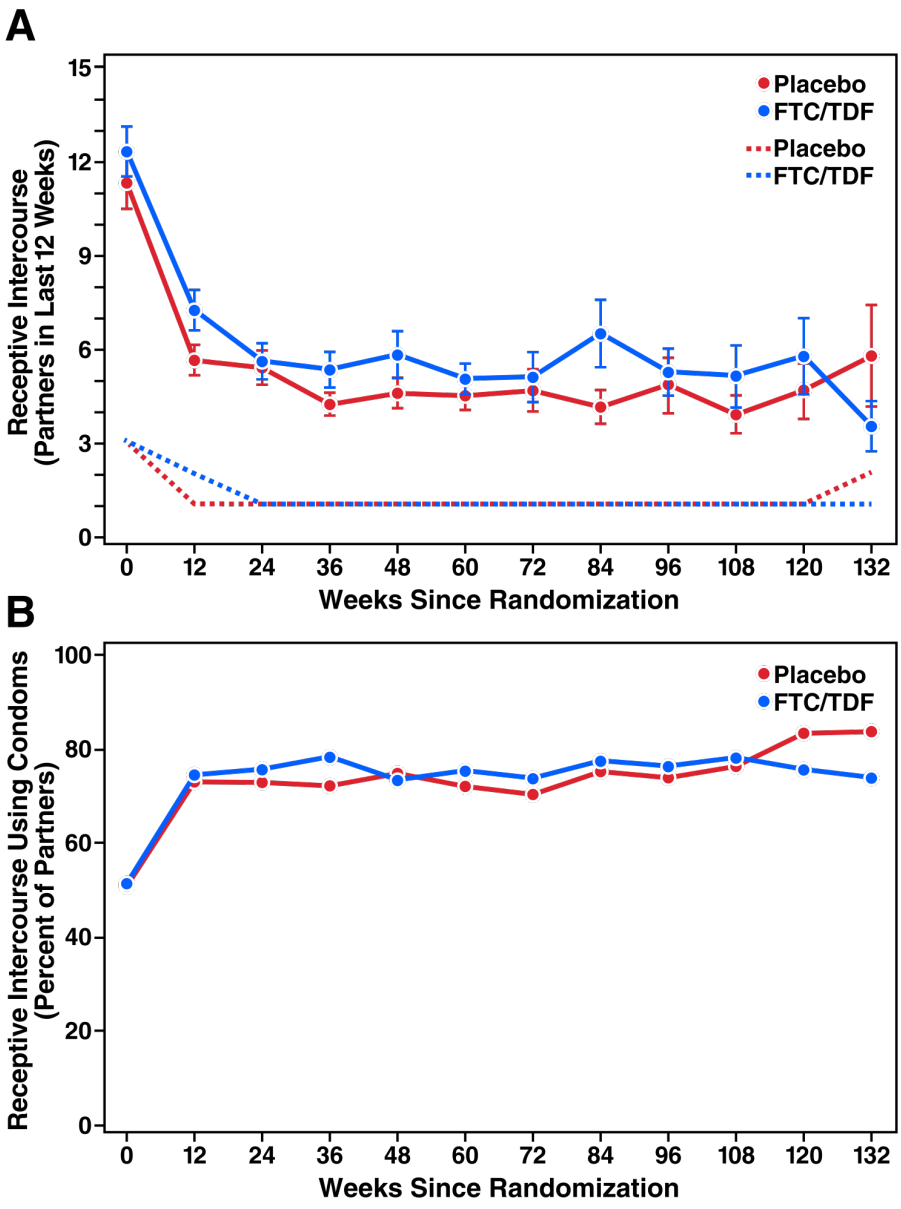
This information also appears in Table 1 of the article, and is reproduced here to specify the serological patterns used for each of the categories.

***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.***



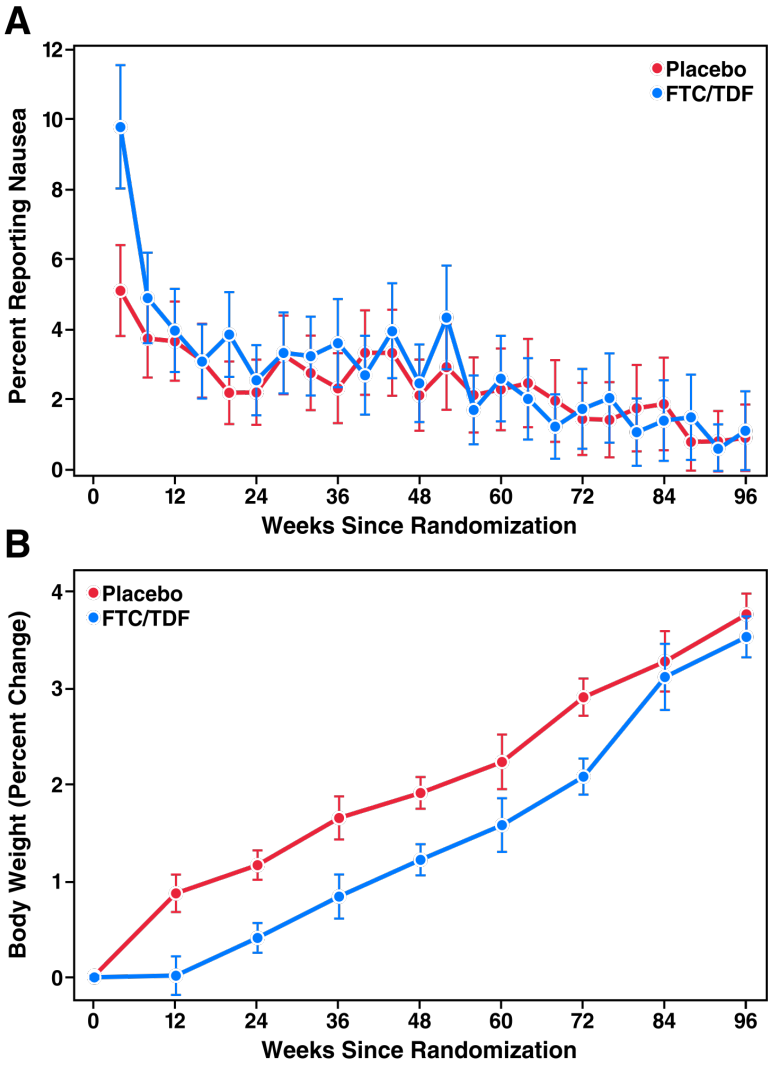

Partners with whom the participant had receptive anal sex in the previous 12 weeks (Panel A), and percentage of those partners using a condom (Panel B) by time on study and group. In Panel A, solid lines represent means and dotted lines represent median numbers, and the error bars are the standard error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

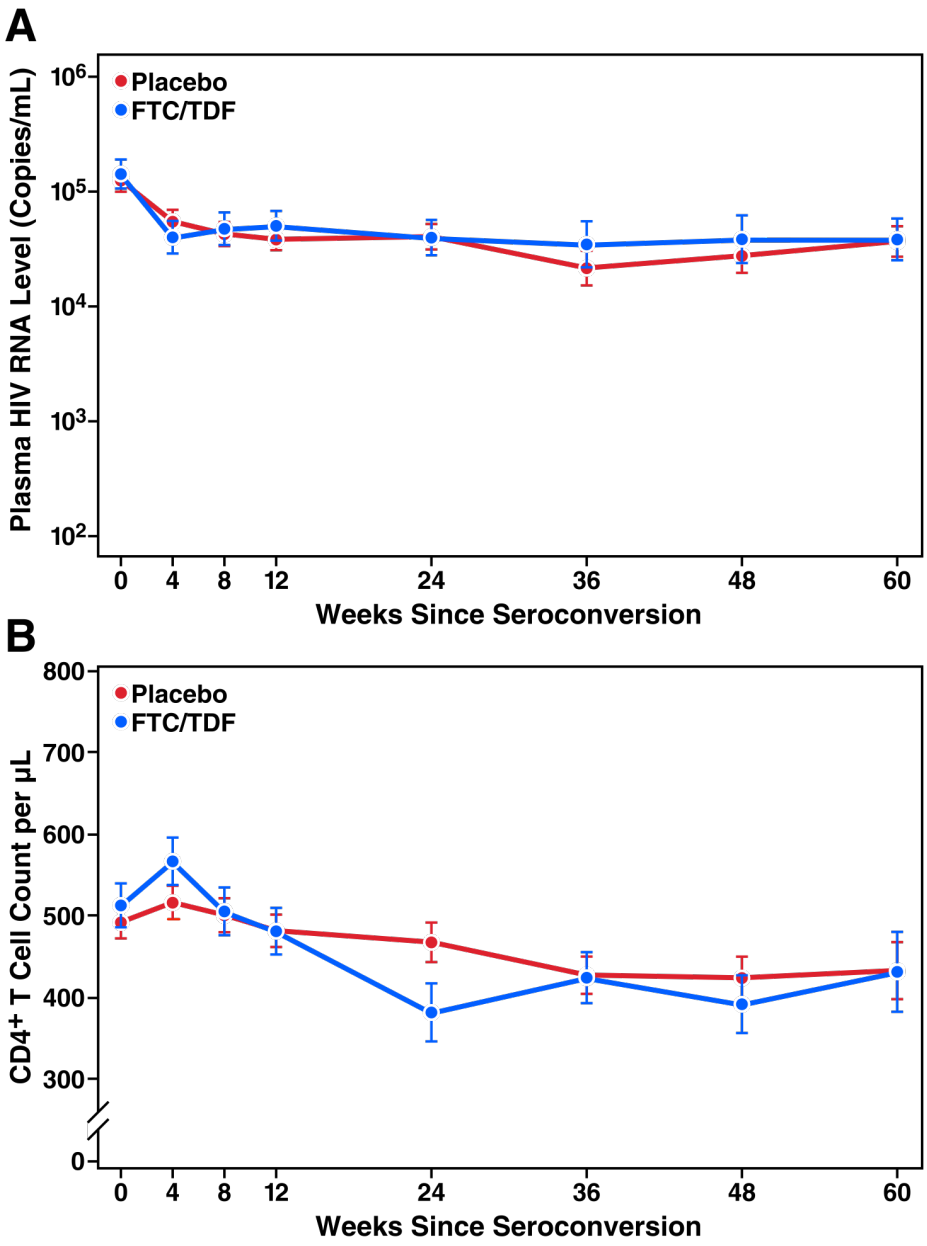
Medical examinations were performed at least every 24 weeks and laboratory testing for sexually transmitted infections (STIs) was performed every 24 weeks regardless of whether symptoms were reported. Gonorrhea and chlamydia PCR was performed if the urine leukocyte esterase was positive. P-values were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



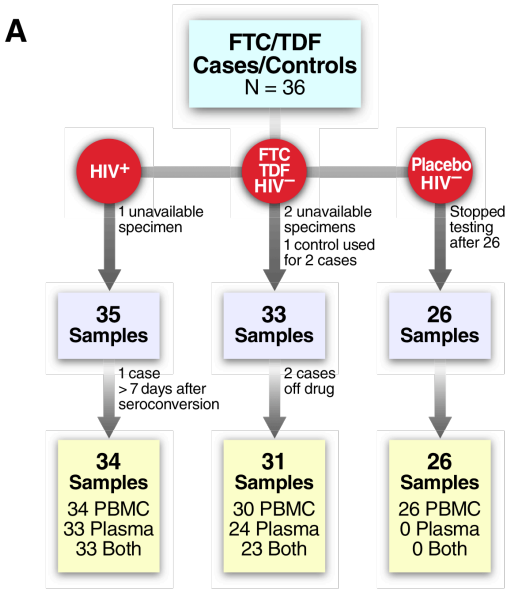

Plasma RNA level at the seroconversion visit was comparable in the two groups (5.15 versus 5.10 log10 copies/ml in the FTC/TDF and placebo groups respectively, P=0.72). At that visit, median reported pill use in the FTC/TDF group was 100% among respondents, of whom 25% reported pill use on less than 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***




C

| | 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.[19,27,28] 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,[29] 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.[30] 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.[31] 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.[32] 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.[33]

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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