*Study Schema:*

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*Hepatitis B immunity criteria for eligibility*

Eligibility required having no detectable hepatitis B surface antigen. Participants at the Bangkok site were required to be immune to hepatitis B either from prior infection or vaccination, which was offered as part of the study. Based on emerging information regarding PrEP safety in the presence of HBV infection [1, 2], participants who were susceptible to hepatitis B in Harlem were eligible for the study and were offered hepatitis B vaccination.

*Randomization and masking*

At week 6 of study participation, after completing an initial one tablet once per week directly observed dosing phase, eligible participants were randomly assigned to one of three dosage groups in a 1:1:1 ratio: daily dosing (one FTC/TDF tablet per day), time-driven dosing (one FTC/TDF tablet taken twice per week, plus one post-exposure FTC/TDF tablet taken within 2 hours after sex), and event-driven dosing (one FTC/TDF tablet within 48 hours prior to sex and another FTC/TDF tablet taken within 2 hours after sex). Site staff logged into a password-protected randomization system and entered the participant’s study code; and the random assignment was shown on the screen. Randomization was stratified by site and used random block sizes of 6, 9, or 12. The randomization code was computer generated by the HPTN Statistical Data Monitoring Center at the Fred Hutchison Research Center at the University of Washington. Laboratory investigators involved in measuring drug concentrations and confirmatory tests for HIV infection were blinded to study arm; participants and all other investigators and staff were not blinded to study arm assignment.

*Laboratory procedures*

Testing for HIV, HBV, and safety (complete blood count, creatinine, phosphate, aspartate aminotransferase [AST], and alanine aminotransferase [ALT]) were performed at screening at the study sites. Safety testing was repeated at weeks 4, 10, 18 and 30. Two HIV rapid tests were performed in parallel at study sites; tests used included the Unigold Recombigen HIV test (Trinity Biotech PLC, Bray, County Wicklow, Ireland); the Determine HIV-1/2 Ag/Ab Combo test (Abbott Laboratories, Abbott Park, IL); and the OraQuick Advance Rapid HIV-1/2 Antibody Test (Orasure Technologies Inc.,Bethlehem, PA). If one or both rapid tests were reactive, a Western blot or the Aptima HIV-1 RNA qualitative assay (Hologic) was performed; this testing was performed using a second sample collected on the same day to confirm infection. A separate second sample was drawn and tested for HIV to confirm infection. Blood samples were collected at each visit. Site laboratories prepared and stored plasma, peripheral blood mononuclear cell (PBMC) lysate, and dried blood spots (DBS, Harlem site only).

Additional testing was performed at the HPTN Laboratory Center (Johns Hopkins University, Baltimore, MD). This included confirmation of all seroconversion events; HIV RNA testing was performed using samples from the visit prior to seroconversion to detect acute HIV infection. Samples from the last study visit were tested using the Abbott Architect HIV ½ Combo (Abbott) to confirm that participants who were not identified as seroconverters were uninfected. A clinical drug resistance genotyping assay (Viroseq, Abbott) was performed using plasma from the first seropositive visit. Plasma, PBMC lysate, and DBS were tested for study medications. Results were not obtained for PBMC lysate samples from the Harlem site because of technical problems in specimen preparation at the study site. Plasma concentrations of TFV and FTC were determined via a validated liquid chromatographic-tandem mass spectrometric (LC-MS/MS) method [3]. The lower limits of quantification for both NRTIs are 0.31 ng/mL. Intracellular tenofovir-diphosphate (TFV-DP) in red blood cell (DBS) and PBMC lysate specimens was quantified at the University of Colorado using a validated LC-MS/MS method [4, 5]. A 3mm punch from DBS and approximately 9 million PBMCs were assayed. The lower limits of quantification were 25 fmol/sample for DBS and 2.5 fmol/sample for PBMC lysate.

*Counseling*

Counseling was based on methods used in Next Step Counseling [6, 7]. Participants in the daily arm received a recommendation to take one tablet once a day regardless of sexual activity. Participants in the time-driven arm received recommendations to take one tablet two days per week, three to four days apart, regardless of sexual activity. In addition, they were asked to take a post-exposure dose of one tablet within two hours after sexual intercourse, defined as any penile intromission, whether oral, vaginal, or anal, and regardless of condom use. Participants in the event-driven arm received a recommendation to take one tablet between 24 to 48 hours prior to sexual intercourse, and a second post-exposure dose within two hours of sexual events (defined as above). Participants in all randomization groups were advised to take a dose if they ever recognized they had missed a dose, provided they took no more than 1 dose in any 2 hour period, no more than 2 doses per day, and no more than seven tablets per week.

*Detailed outcome definitions and measurements*

Secondary aims included adherence to the recommended regimen (defined as the number of tablets taken on time (according to study arm instructions) divided by the numbers recommended). While the definition of coverage of sex events was identical in all randomization groups, the definition of adherence differed by group. Adherence in the daily arm was defined as one tablet taken per day. Adherence in the time-driven arm was defined as one tablet taken at least every 4 days and an additional tablet taken within 24 hours after sex (including oral sex). Adherence in the event-driven arm was defined as one tablet taken within 48 hours before sex and another one tablet taken within 24 hours after sex (including oral sex). Other secondary aims include side effects, regimen switching, HIV seroconversions, drug resistance, and sexual practices. The study was planned to evaluate the primary outcomes at each study site separately, based on the premise that social and cultural differences between sites could affect PrEP-related behavior.

*Power calculations*

The study was designed to have 90% power to test the noninferiority null hypothesis that the difference in the proportion of uncovered acts between either non-daily arm and the daily arm was >0.031, versus the alternative that the difference was less than 0.031, assuming 1) 60 participants per arm (i.e. each site powered separately), 2) the proportion of uncovered acts in the daily arm would be 0.10, 3) participants would report an average of 50 acts over follow-up (~ 2/week), 4) the inter-person coefficient of variation in coverage was 0.40, and 5) one-tailed α = 0.05 with no adjustment for multiple testing.

*Trial monitoring*

The trial was monitored by a data safety and monitoring board. The only stopping rule was based on HIV infections—if excess HIV infections were observed in the non-daily arms compared to the daily arm, the trial would be stopped. The protocol was approved by the Ethical Review Committee for Research in Human Subjects of the Thailand Ministry of Public Health, and by Institutional Review Boards of the U.S. Centers for Disease Control and Prevention and Columbia University Medical Center. All analyses were done using SAS version 9.4.

*References cited in supplemental material*

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