Dose Response for Starting and Stopping HIV Preexposure Prophylaxis for Men Who Have Sex With Men

Sharon M. Seifert,[1] David V. Glidden,[2] Amie L. Meditz,[3] Jose R. Castillo-Mancilla,[4] Edward M. Gardner,[4,5] Julie A. Predhomme,[1] Caitlin Rower,[1] Brandon Klein,[1] Becky J. Kerr,[1] L. Anthony Guida,[1] Jia-Hua Zheng,[1] Lane R. Bushman,[1] and Peter L. Anderson[1]

[1]Skaggs School of Pharmacy and Pharmaceutical Sciences Department of Pharmaceutical Sciences, University of Colorado Denver, Aurora; [2]Department of Epidemiology and Biostatistics, University of California, San Francisco; [3]Beacon Center for Infectious Disease, Boulder Community Hospital, [4]School of Medicine, Division of Infectious Diseases, University of Colorado Denver, Aurora, and [5]Denver Public Health, Colorado

Background. This study estimated the number of daily tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) doses required to achieve and maintain (after discontinuation) intracellular drug concentrations that protect against human immunodeficiency virus (HIV) infection for men who have sex with men (MSM).

Methods. Tenofovir diphosphate (TFV-DP) concentrations in peripheral blood mononuclear cells (PBMCs) and rectal mononuclear cells from an intensive pharmacokinetic study ("Cell-PrEP" [preexposure prophylaxis]) of 30 days of daily TDF/FTC followed by 30 days off drug were evaluated. A regression formula for HIV risk reduction derived from PBMCs collected in the preexposure prophylaxis initiative study was used to calculate inferred risk reduction. The time required to reach steady state for TFV-DP in rectal mononuclear cells was also determined.

Results. Twenty-one HIV-uninfected adults participated in Cell-PrEP. The inferred HIV risk reduction, based on PBMC TFV-DP concentration, reached 99% (95% confidence interval [CI], 69%–100%) after 5 daily doses, and remained >90% for 7 days after stopping drug from steady-state conditions. The proportion of participants reaching the 90% effective concentration (EC_{90}) was 77% after 5 doses and 89% after 7 doses. The percentage of steady state for natural log [TFV-DP] in rectal mononuclear cells was 88% (95% CI, 66%–94%) after 5 doses and 94% (95% CI, 78%–98%) after 7 doses.

Conclusions. High PrEP activity for MSM was achieved by approximately 1 week of daily dosing. Although effective intracellular drug concentrations persist for several days after stopping PrEP, a reasonable recommendation is to continue PrEP dosing for 4 weeks after the last potential HIV exposure, similar to recommendations for postexposure prophylaxis.

Keywords. preexposure prophylaxis; HIV; tenofovir; pharmacokinetics; MSM.

New human immunodeficiency virus (HIV) infections have increased among young minority men who have sex with men (MSM) in recent years [1]. Preexposure prophylaxis (PrEP) with daily tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) provides an important tool for preventing HIV infection in MSM as well as heterosexual adults at risk for exposure to HIV [2–7]. Several guidelines for PrEP have been published; however, there is a lack of recommendations regarding how to start and stop PrEP relative to potential HIV exposures. Therefore, studies are needed that estimate the onset and duration of high PrEP activity (eg, >90% efficacy).

Knowledge of the pharmacokinetic/pharmacodynamic (PK/PD) profile for PrEP is required to estimate the onset and duration of PrEP activity. Such information was generated using data from the preexposure prophylaxis initiative (iPrEx) trial, a large, randomized placebo controlled study that enrolled 2499 transgender

Received 8 September 2014; accepted 7 November 2014; electronically published 18 November 2014.

Correspondence: Peter L. Anderson, PharmD, Department of Pharmaceutical Sciences, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, V20-C238, Rm 4101, 12850 E Montview Blvd, Aurora, CO 80045 (peter.anderson@ucdenver.edu).

Clinical Infectious Diseases® 2015;60(5):804–10

DOI: 10.1093/cid/ciu916

women and MSM [8, 9]. A post hoc regression analysis identified a continuous relationship between HIV risk reduction (relative to placebo) and intracellular tenofovir diphosphate (TFV-DP) concentration, the pharmacologically active moiety for tenofovir, in viable cryopreserved peripheral blood mononuclear cells (vPBMCs). A benchmark in the continuous relationship was that a TFV-DP concentration of 16 (95% confidence interval [CI], 3–28) fmol/10^6 cells was associated with a 90% reduction in HIV acquisition [10]; this was referred to as the EC_{90} (ie, the 90% effective concentration). The objective of the present study was to apply the regression formula derived from iPrEx to PBMC TFV-DP concentrations from an intensive pharmacokinetic study in HIV-uninfected persons (Cell-PrEP) to estimate HIV risk reduction per TDF/FTC dose.

METHODS

Intensive Pharmacokinetic Study Design

Cell-PrEP was a prospective, observational, pharmacokinetic study in HIV-uninfected adult male and female volunteers aged 18–55 years, conducted at the University of Colorado Denver, Anschutz Medical Campus (ClinicalTrials.gov identifier NCT01040091). The study was approved by the institutional review board and participants provided written informed consent prior to participating. Subjects received daily TDF 300 mg/FTC 200 mg (as Truvada) for 30 days, followed by 30 days off drug; the total study duration was 60 days.

PBMCs were collected on days 1, 3, 7, 20, 30, 35, 45, and 60. On days 1 and 30, blood was collected at 1-, 2-, 4-, 8-, and 24-hour intervals postdose; on days 3, 7, and 20, blood was collected predose and at 2 and 8 hours postdose. Subjects were asked to fast overnight, beginning at 10 PM, prior to their dosing visits (days 1–30). Rectal biopsy samples were collected once for each subject at 2 hours postdose at one of their dosing visits. These collections were staggered such that 4 participants had the rectal biopsy for each of the 5 dosing visits (days 1, 3, 7, 20, and 30). For the washout phase, blood was collected on days 35, 45, and 60. A window of several days was allowed around all scheduled visits. Adherence was determined by pill count, self-report, and a dosing calendar on which participants recorded dosing times.

Freshly Lysed PBMC Processing

PBMCs were harvested from cell preparation tubes with standard laboratory procedures. Red blood cells (RBCs) were lysed with RBC lysis buffer followed by rinsing and automated cell counting (Countess, Invitrogen). Cells were lysed in 500 µL methanol: water ($MeOH:H_2O$) and stored at –80°C until analysis.

Viable Cryopreserved PBMC Processing

At the day 7 visit, a vPBMC sample was collected along with the freshly lysed PBMC sample (as described above) at the predose time point. The vPBMC sample was processed using a freezing solution of Roswell Park Memorial Institute medium (RPMI), fetal bovine serum, and dimethyl sulfoxide, and the viable cells were stored at –80°C until processing for the assay. Just prior to assaying, the frozen PBMC samples were thawed and processed with the same procedures that were described previously for the iPrEx analysis [8, 10].

Rectal Mononuclear Cell Isolation

Approximately 20 pinches of rectal tissue were collected by a gastroenterologist during sigmoidoscopy from 4 to 12 inches into the rectum using a 3-mm forceps. Biopsies were placed into 30 mL of phosphate-buffered saline and washed with complete RPMI, followed by 2–3 digestions with Collagenase solution at 37°C for 30 minutes, with gentle agitation. The cell suspension was collected by straining the digested solution through a cell strainer. After RBC lysis, the remaining mononuclear cells were washed, counted, assessed for viability, lysed in 500 µL of $MeOH:H_2O$, and stored at −80°C until analysis.

Drug Assay

TFV-DP and emtricitabine-triphosphate (FTC-TP) concentrations in lysed cellular matrices were assayed with a validated liquid chromatography–tandem mass spectrometry method, as described previously [11]. The lower limit of quantification (LOQ) was 2.5 fmol/sample for TFV-DP and 0.1 pmol/sample for FTC-TP. This was the same analytical procedure used previously to assay iPrEx samples [8].

Pharmacokinetic Analysis

All TFV-DP and FTC-TP concentrations in PBMCs from all visits in all participants were used for the pharmacokinetic model. The pharmacokinetic concentration–time profiles for each individual were characterized using a 1-compartment constant drug input model with Phoenix WinNonlin. Rectal mononuclear cell concentrations were characterized with the same model using GraphPad Prism version 6.00 (GraphPad Software, La Jolla, California) with least squares regression, but using a naive-pooled approach.

Data Analysis

Freshly lysed PBMC TFV-DP concentrations from Cell-PrEP were converted to vPBMC values for use in the iPrEx regression formula [10], because the iPrEx regression formula was generated using TFV-DP from vPBMC, and the samples from Cell-PrEP were freshly lysed PBMCs. Multiple imputation was used for inferring the TFV-DP values in vPBMCs. The multiple imputation used data from 65 paired viable-fresh values, including some from HIV-infected individuals. Each predicted viable cryopreserved TFV-DP value was the result of 20 imputations that accounted for variability. Sensitivity analyses were conducted using multiple imputation with the following reduced datasets for viable-fresh values: removing values from viable cryopreserved samples in short-term storage (≤3 months), removing values from HIV-infected persons, removing values from women in Cell-PrEP, and removing values from those in Cell-PrEP with known missed doses. Finally, the raw TFV-DP concentrations in vPBMC from the day 7 visit of Cell-PrEP were directly applied to the iPrEx regression formula (n = 20). These TFV-DP results corresponded to the predose concentration on days 5, 6, or 7, depending on when the participant came in during their day 7 visit window. This included 1 vPBMC result that showed no signal for TFV-DP (or FTC-TP) despite a high value in the freshly lysed sample (greater than the 75th percentile of freshly lysed values). A TFV-DP value of "0" was included for this sample.

The mean log vPBMC values were modeled using a linear mixed effects model with a cubic spline fit to days since starting daily TDF/FTC. Three approaches were used to estimate the onset and duration of PrEP activity. First, the inferred HIV risk reduction was calculated using estimated TFV-DP concentrations (in vPBMCs) from Cell-PrEP and analyzed with a previously described regression equation from iPrEx [10], for each dose of daily TDF/FTC, as well as each day after TDF/FTC dosing was stopped. Second, the proportion of Cell-PrEP individuals with TFV-DP concentrations greater than or equal to the iPrEx EC_{90} (16 fmol/10^6 vPBMCs) was determined over the same time frame. Finally, to evaluate corresponding drug concentrations at an important site of drug action in MSM, the time that TFV-DP concentrations reached steady state in rectal mononuclear cells was determined.

RESULTS

Subject Characteristics

Twenty-one HIV-uninfected men and women were enrolled in Cell-PrEP, and 19 completed all study visits. Of the 2 participants who did not complete all study visits, 1 had grade II low phosphorus and was removed from the study per protocol, while the other participant chose to withdraw for personal reasons. Nineteen rectal samples were collected and analyzed from 19 participants, and 410 PBMC samples were analyzed from 21 participants. The demographics of the study participants are shown in Table 1. A total of 5 missed doses (out of 584 doses) were reported by participants in the study, supported by pill counts, indicating an overall adherence of 99%.

Table 1. Overall Demographics and Baseline Characteristics (N = 21)

Characteristic	No. (%) or Median (Range)
Age, y	31 (20–47)
Sex	
Male	11 (52.4)
Female	10 (47.6)
Race/ethnicity	
White	10 (47.6)
African American	10 (47.6)
Hispanic	1 (4.8)
Weight, kg	81.1 (56.5–124.4)
BMI, kg/m^2	27.2 (20.8–37.7)
eGFR, mL/min/1.73 m^2	93.3 (67.8–128.6)

Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate.

Inferred HIV Risk Reduction

The inferred HIV risk reduction based on PBMC TFV-DP concentration was 77% (95% CI, 40%–93%) after 1 dose, 96% (95% CI, 60%–100%) after 3 doses, 99% (95% CI, 69%–100%) after 5 doses, and 99% (95% CI, 70%–100%) after 7 doses (Figure 1). The inferred risk reduction did not reach 100% during the study. The corresponding inferred risk reduction from the sensitivity analyses ranged from 75% to 81% after 1 dose, 95% to 97% after 3 doses, and 98% to 99% after 5 and 7 doses. Analysis of the raw TFV-DP concentrations from the day 7 visit (predose from doses 5, 6, or 7), with and without the sample with no drug detection, revealed a 92% and 96% risk reduction, respectively. After dosing was discontinued at day 30, the inferred risk reduction remained >90% for 7 days. In sensitivity analyses, after stopping drug for 7 days from day 30, the risk reduction ranged from 90% to 94%.

Proportion Above the iPrEx EC_{90}

The proportion of PBMC TFV-DP concentrations that reached the iPrEx EC_{90} was 30% after 1 dose, 55% after 3 doses, 77% after 5 doses, 89% after 7 doses, and 98% after the 13th dose (Figure 1). The proportion reaching the iPrEx EC_{90} did not reach or exceed 99% during the study. The corresponding values from the sensitivity analyses ranged from 26% to 41% after 1 dose, 53% to 67% after 3 doses, 78% to 85% after 5 doses, 91% to 95% after 7 doses, and 99% after the 13th dose. Analysis of the raw TFV-DP concentrations from the day 7 visit (predose from doses 5, 6, or 7), with and without the sample with no drug detection, revealed 85% and 89% above the iPrEx EC_{90}, respectively. After stopping drug, 80% of TFV-DP concentrations remained above the iPrEx EC_{90} for 2 days, decreasing to 48% at 7 days postdrug discontinuation. In sensitivity analyses, the proportion of concentrations above the EC_{90} ranged from 86% to 91% at 2 days after stopping drug, and 48% to 63% at 7 days after stopping drug.

Rectal Mononuclear Cell Concentrations

Seventeen of the 19 rectal samples had quantifiable TFV-DP concentrations, whereas 2 samples had concentrations below

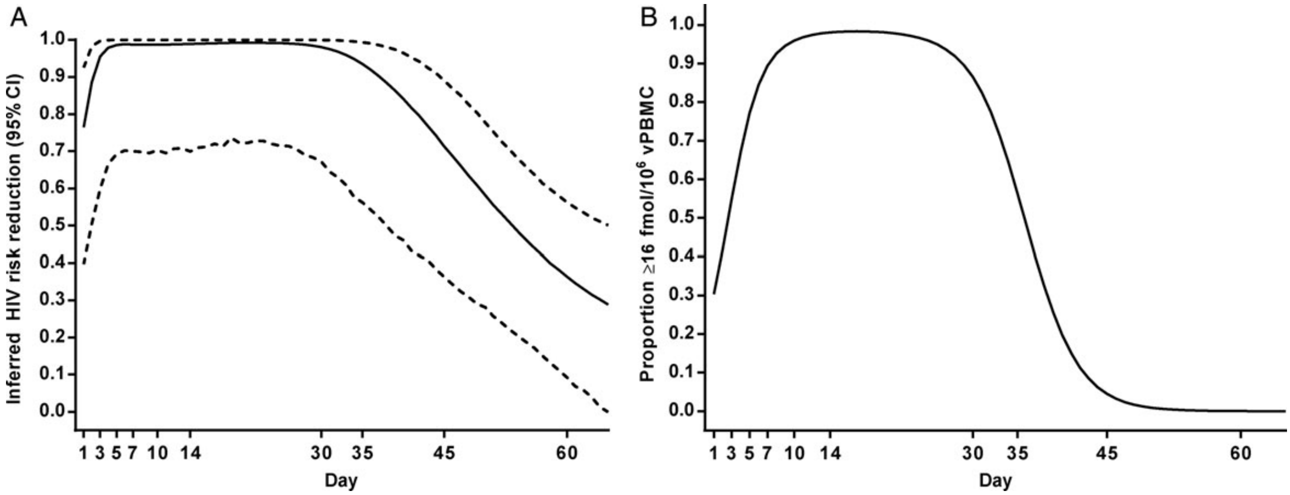

Figure 1. Inferred human immunodeficiency virus (HIV) risk reduction and proportion ≥90% effective concentration (EC_{90}). *A*, Study day is depicted on the x-axis; inferred HIV risk reduction is depicted on the y-axis. The solid line represents the point estimate for reduced risk for HIV infection; the dashed lines represent the 95% confidence interval (CI). *B*, Proportion of participants with tenofovir diphosphate (TFV-DP) concentrations in viable cryopreserved peripheral blood mononuclear cells (vPBMCs) at or above the preexposure prophylaxis initiative (iPrEx) (16 fmol/10^6cells) is depicted on the y-axis. The solid line represents the proportion of participants with TFV-DP concentrations at or above the iPrEx EC_{90} by day in the study.

the LOQ, both from the first-dose visit. One of the 19 FTC-TP concentrations was below the LOQ, from the day 3 visit. A value midway between 0 and the lower LOQ was used for these concentrations in all calculations. TFV-DP concentrations were natural log (ln) transformed prior to fitting the pharmacokinetic model (Figure 2). The half-life of rectal mononuclear cell TFV-DP (ln) was 1.7 (95% CI, 1.2–3.2) days and the steady-state concentration (ln) was 7.6 (95% CI, 6.8–8.4) fmol/10^6cells, corresponding to 1961 (95% CI, 854–4501) fmol/10^6cells after back-transformation. The half-life based on this model predicted that 71% (95% CI, 48%–82%) of steady-state was reached after 3 doses, 88% (95% CI, 66%–94%) after 5 doses, and 94% (95% CI, 78%–98%) after 7 doses. The steady-state FTC-TP concentration in rectal mononuclear cells was 0.96 (95% CI, 0.53–1.4) pmol/10^6cells, but the half-life could not be accurately estimated (1.5 [95% CI, 0.5–∞] days) (Figure 2).

PBMC Pharmacokinetics

The 2 participants who withdrew early were not included in the pharmacokinetic modeling for PBMCs, but their data were included in the inferred risk reduction analyses described above. Using the concentrations from dosing visits, the mean TFV-DP steady-state concentration was 103 fmol/10^6cells (coefficient of variation [CV], 32%) and the mean half-life was 3.5 days (CV, 31%) in the 19 participants who completed all study visits (Figure 3). Eighteen percent of steady-state was achieved after 1 dose, 45% after 3 doses, 63% after 5 doses, 75% after 7 doses, and >90% after 12 doses. The mean FTC-TP steady-state concentration was 6 pmol/10^6cells (CV, 22%) and the mean half-life was 33 hours (CV, 42%) (Figure 3). Forty percent of steady state was achieved after 1 dose, 78% after 3 doses, 92% after 5 doses, and 97% after 7 doses. There were no differences between men and women in steady-state TFV-DP ($P = .46$) or FTC-TP ($P = .69$) concentrations.

DISCUSSION

This study evaluated pharmacokinetic data from daily dosing in Cell-PrEP with a pharmacodynamic model for HIV risk reduction from iPrEx, and calculated an inferred HIV risk reduction that reached 99% by 5 days of daily dosing. This 99% value provides an average estimate based on the set of TFV-DP concentrations from Cell-PrEP participants for each daily dose, suggesting that most concentrations conferred nearly 100% efficacy from the fifth dose onward. Sensitivity analyses, including one in which data from female participants were excluded, showed consistent results.

A second, more conservative approach, showed that the majority of Cell-PrEP participants exceeded the iPrEx EC_{90} by the fifth or seventh doses, approximately 80% and 90%, respectively, and that the maximum proportion was achieved by the 13th dose (98%). Reaching the highest proportion by the 13th dose was consistent with the pharmacokinetic profile of TFV-DP in PBMCs, which demonstrated that >90% steady state was achieved after approximately 12 doses (Figure 3). FTC-TP in PBMCs achieved steady state more rapidly—92% after 5 doses and 97% after 7 doses.

A third approach focused on TFV-DP (ln transformed) at an important site of action in MSM, in rectal mononuclear cells, showing that 88% and 94% of steady state were achieved after

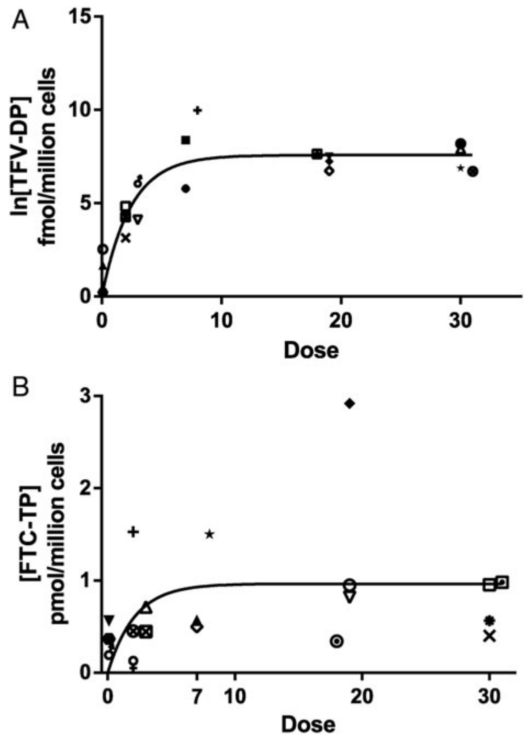

Figure 2. Tenofovir diphosphate (TFV-DP) and emtricitabine-triphosphate (FTC-TP) in rectal mononuclear cells. *A*, Concentrations of TFV-DP in rectal mononuclear cells were first natural log (ln) transformed and then fit using a nonlinear 1-phase exponential association [$Ct = Css \times (1\text{-}e^{-K \times t})$], where Ct is the (ln) concentration at time t, Css is the fitted (ln) steady-state drug concentration, and K is the fitted first-order elimination rate constant. *B*, FTC-TP concentrations (not ln transformed) in rectal mononuclear cells were fit with the same nonlinear 1-phase exponential association.

5 and 7 doses, respectively (Figure 2). FTC-TP concentrations in rectal mononuclear cells also appeared to reach steady state by this time.

Taken together, a high level of PrEP activity was achieved by 5–7 doses, indicating that, for optimal benefit, PrEP should be started approximately 1 week before it is needed. It is important to note that this analysis was based on data in MSM (iPrEx), so these results should not be extrapolated to women, heterosexual men, or other routes of transmission. Understanding and applying PK/PD analyses for PrEP in women, and populations other than MSM, is an urgent research priority.

Other studies also support approximately 1 week of lead-in time for starting PrEP in MSM. The HIV Prevention Trials Network (HPTN) 066 study, which used directly observed dosing for 5 weeks to characterize TFV-DP and FTC-TP in PBMCs among 45 HIV-uninfected men and women, showed that steady state was achieved by 7 days for both TFV-DP and FTC-TP [12]. An advantage of HPTN 066 compared with Cell-PrEP was the directly observed dosing design. Reaching steady state by 7 days indicates that inferred HIV risk reduction and proportion above the iPrEx EC_{90} would plateau by day 7. Another study with consistent results was the iPrEx Open Label Extension, which showed that no HIV infections were observed in MSM when PrEP dosing was estimated to be ≥4 doses per week (HIV risk reduction, 100% [95% CI, 84%–100%]) [13], corresponding to greater than four-sevenths (57%) of steady-state concentrations. This level of steady state (57%) would be achieved in the present study after 4 doses using TFV-DP kinetics in PBMCs and 2 doses in rectal mononuclear cells. Finally, the Ipergay study evaluated placebo vs 2 TDF/FTC tablets before anticipated sex followed by 1 tablet 24 and 48 hours after sex among MSM [14]. An unblinded analysis by the data safety monitoring board showed that a significant reduction in HIV incidence (magnitude not reported) was observed in the TDF/FTC vs placebo group. Together these observations support high PrEP activity by approximately 1 week for MSM [15].

The considerations for how to safely stop PrEP after the last potential HIV exposure are not as straightforward. The present study found that high PrEP activity was evident for several days after dosing was stopped. The inferred risk reduction exceeded 90% for 7 days, and 80% of participants remained above the iPrEx EC_{90} for 2 days after stopping PrEP. However, an important consideration for discontinuing PrEP is how long it takes for HIV to be completely cleared from the body following the last potential exposure. There appears to be little consensus on this issue given that several variables may affect the HIV clearance process, such as whether HIV is endocytosed into Langerhans cells (where it may persist for days), and/or adhered to follicular dendritic cells, and/or whether early cycles of HIV replication occur [16, 17]. As early stages of viral replication occur, longer times are required to completely clear the virus, and the chance for establishing latent infection increases. This was demonstrated in early postexposure prophylaxis (PEP) studies in animals that showed 50% vs 100% efficacy for 10 days vs 28 days of tenofovir dosing when started 24 hours after intravenous simian immunodeficiency virus exposure [16]. These considerations underlie the current PEP recommendations to treat potential HIV exposures for 28 days [18].

PrEP differs from PEP in that early replication is presumably blocked by PrEP, as long as PrEP activity is high, perhaps allowing for a faster HIV clearance rate in the setting of PrEP. Similar efficacy was found in animal studies that compared continued PrEP dosing for 28 days after the last viral inoculation vs discontinued dosing after the last viral inoculation [19]. These considerations suggest that shorter durations of dosing might be adequate for PrEP following the last potential HIV exposure, but not enough information is available to make specific recommendations. This should be an area of future research. Until then, a conservative recommendation would be to continue dosing for 4 weeks after the last potential HIV exposure.

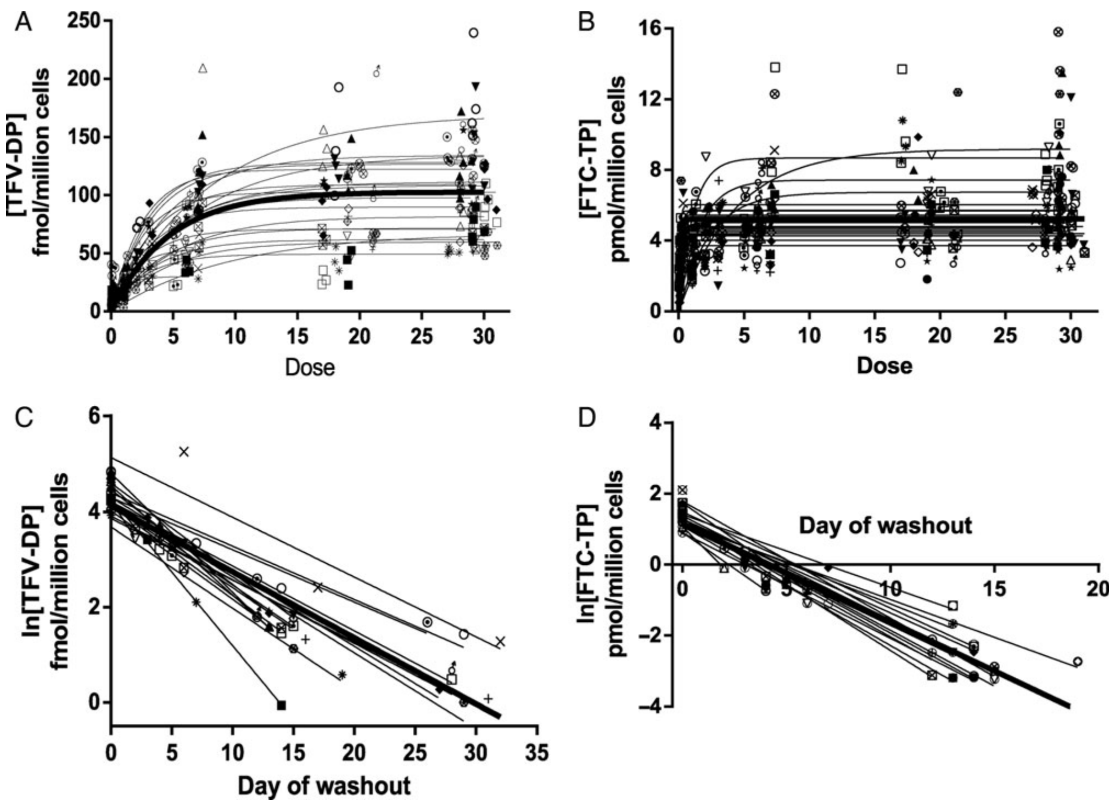

Figure 3. Tenofovir diphosphate (TFV-DP) and emtricitabine-triphosphate (FTC-TP) concentration vs time curves in freshly lysed peripheral blood mononuclear cells (PBMCs). All TFV-DP concentrations from all visits were included in the graphs for all but 2 participants (who withdrew from the study early). All FTC-TP concentrations from all study visits were included. The graphs depict the accumulation phase of TFV-DP (*A*) and FTC-TP (*B*) as well as the washout phase of TFV-DP (*C*) and FTC-TP (*D*) in freshly lysed PBMCs. For the washout phase, both TFV-DP and FTC-TP concentrations were natural log (ln) transformed prior to fitting with a linear regression.

The strengths of this study include the several lines of evidence used to estimate PrEP efficacy for each daily dose, including use of a PK/PD model from iPrEx to analyze drug concentrations from an intensive pharmacokinetic study, Cell-PrEP, with all drug concentrations processed and assayed with the same laboratory procedures; analysis of drug accumulation at an important site of action for MSM; and the consistency of the findings from this study with other studies. The main limitations include that the onset and duration of action could not be studied directly in vivo, and that these results were based upon studies in MSM, so the findings cannot be extrapolated to other modes of transmission such as vaginal, penile (heterosexual), or parenteral. Additionally, other HIV transmission characteristics may be important to the PK/PD profile, but were not considered here, such as the viral inoculum size [20]. Last, adherence in Cell-PrEP was monitored using self-report and pill count, which may not have accurately reflected true dose-taking behavior among participants [21].

In summary, this study indicates that approximately 1 week of daily PrEP is expected to confer high PrEP activity for MSM. Although a high level of protection may persist for several days after stopping PrEP from steady state, 4 weeks of continued PrEP dosing is reasonable relative to the last potential HIV exposure.

Notes

Acknowledgments. We thank the study participants and staff of the Colorado Clinical Translational Research Center (CTSI).

Disclaimer. The contents are the authors' sole responsibility and do not necessarily represent the official views of the National Institutes of Health (NIH).

Financial support. This work was supported by NIH/National Center for Advancing Translational Sciences Colorado CTSI (grant number UL1 TR001082) and the National Institute of Allergy and Infectious Diseases (grant number U01 AI84735). Study drug was donated by Gilead Sciences.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Centers for Disease Control and Prevention. HIV/AIDS surveillance overview: HIV incidence. Available at: http://www.cdc.gov/hiv/statistics/surveillance/incidence/. Accessed 24 November 2014.

2. Anderson PL, Kiser JJ, Gardner EM, Rower JE, Meditz A, Grant RM. Pharmacological considerations for tenofovir and emtricitabine to prevent HIV infection. J Antimicrob Chemother **2011**; 66:240–50.
3. Centers for Disease Control and Prevention. Interim guidance: preexposure prophylaxis for the prevention of HIV infection in men who have sex with men. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6003a1.htm/. Accessed 24 November 2014.
4. Centers for Disease Control and Prevention. Update to interim guidance for preexposure prophylaxis (PrEP) for the prevention of HIV infection: PrEP for injecting drug users. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6223a2.htm. Accessed 24 November 2014.
5. Centers for Disease Control and Prevention. Interim guidance for clinicians considering the use of preexposure prophylaxis for the prevention of HIV infection in heterosexually active adults. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6131a2.htm. Accessed 24 November 2014.
6. US Food and Drug Administration. FDA approves first drug for reducing the risk of sexually acquired HIV infection. **2012**. Available at: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm312210.htm. Accessed 24 November 2014.
7. Centers for Disease Control and Prevention. Preexposure prophylaxis for the prevention of HIV infection in the United States: a clinical practice guideline, **2014**. Available at: http://www.cdc.gov/hiv/pdf/prepguidelines2014.pdf. Accessed 24 November 2014.
8. Grant RM, Lama JR, Anderson PL, et al. Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. N Engl J Med **2010**; 363:2587–99.
9. Grant RM, McMahan V, Liu A, et al. Completed observation of the randomized placebo-controlled phase of iPrEx: daily oral FTC/TDF pre-exposure HIV prophylaxis among men and trans women who have sex with men. In: Sixth International AIDS Society Conference on HIV Pathogenesis, Treatment and Prevention (IAS 2011), Rome, Italy, **2011**.
10. Anderson PL, Glidden DV, Liu A, et al. Emtricitabine-tenofovir concentrations and pre-exposure prophylaxis efficacy in men who have sex with men. Sci Transl Med **2012**; 4:8.
11. Bushman LR, Kiser JJ, Rower JE, et al. Determination of nucleoside analog mono-, di-, and tri-phosphates in cellular matrix by solid phase extraction and ultra-sensitive LC–MS/MS detection. J Pharm Biomed Anal **2011**; 56:390–401.
12. Hendrix C, Andrade A, Kashuba A, et al. Tenofovir-emtricitabine directly observed dosing: 100% adherence concentrations (HPTN 066). In: Conference on Retroviruses and Opportunistic Infections, Boston, MA, **2014**.
13. Grant RM, Anderson PL, McMahan V, et al. Uptake of pre-exposure prophylaxis, sexual practices, and HIV incidence in men and transgender women who have sex with men: a cohort study. Lancet Infect Dis **2014**; 14:820–9.
14. Molina J-M. Good adherence seen in study of intermittent PrEP. In: 20th International AIDS Conference, Melbourne, Australia, **2014**.
15. IPergay press release: a significant breakthrough in the fight against HIV/AIDS. A drug taken at the time of sexual intercourse effectively reduces the risk of infection. Paris, France: France REcherche Nord & Sud Sida-HIV et Hepatites (ANRS), **2014**. Available at: http:/www.anrs.fr/content/download/6008/32756/file/Press%2520release%2520IPERGAY-WEB.pdf. Accessed 24 November 2014.
16. Tsai CC, Emau P, Follis KE, et al. Effectiveness of postinoculation (R)-9-(2-phosphonylmethoxypropyl) adenine treatment for prevention of persistent simian immunodeficiency virus SIVmne infection depends critically on timing of initiation and duration of treatment. J Virol **1998**; 72:4265–73.
17. Hladik F, Sakchalathorn P, Ballweber L, et al. Initial events in establishing vaginal entry and infection by human immunodeficiency virus type-1. Immunity **2007**; 26:257–70.
18. Department of Health and Human Services. Post-exposure prophylaxis. Available at: http://aids.gov/hiv-aids-basics/prevention/reduce-your-risk/post-exposure-prophylaxis/. Accessed 24 November 2014.
19. Garcia-Lerma JG, Otten RA, Qari SH, et al. Prevention of rectal SHIV transmission in macaques by daily or intermittent prophylaxis with emtricitabine and tenofovir. PLoS Med **2008**; 5:e28.
20. Duwal S, Schutte C, von Kleist M. Pharmacokinetics and pharmacodynamics of the reverse transcriptase inhibitor tenofovir and prophylactic efficacy against HIV-1 infection. PLoS One **2012**; 7:e40382.
21. Agot K, Taylor D, Corneli AL, et al. Accuracy of self-report and pill-count measures of adherence in the FEM-PrEP clinical trial: implications for future HIV-prevention trials. AIDS Behav **2014**; doi: 10.1007/s10461-014-0859-z.