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Emtricitabine-Triphosphate in Dried Blood Spots as a Marker of Recent Dosing

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New objective measures of antiretroviral adherence are needed. We determined if emtricitabine triphosphate (FTC-TP) in dried blood spots (DBS) can be used as a marker of recent dosing with tenofovir disoproxil fumarate-emtricitabine (TDF-FTC). The half-life of FTC-TP was estimated in DBS samples obtained from an intensive pharmacokinetic (PK) study of coformulated TDF-FTC in HIV-negative and HIV-infected participants. The concordance of quantifiable FTC-TP in DBS with tenofovir (TFV)/FTC in plasma was evaluated by utilizing paired plasma-DBS samples from participants enrolled in 2 large preexposure prophylaxis (PrEP) open-label trials. The time to FTC-TP nondetectability after TDF-FTC dosing was evaluated utilizing DBS from HIV-negative participants enrolled in a directly observed therapy study of variable adherence to TDF-FTC. The mean (95% confidence interval [CI]) terminal half-life of FTC-TP in the PK study was 35 (23 to 47) h. A total of 143/163 (88%) samples obtained 0 to 48 h post-TDF-FTC dose had quantifiable FTC-TP in DBS, compared with 2/93 (2%) and 0/87 (0%) obtained >48 and >96 h postdose. In 746 paired plasma-DBS samples from 445 participants enrolled in PrEP trials, when both TFV/FTC in plasma were below the limit of quantification, FTC-TP was as well in 98.9% of the samples, and when either TFV or FTC in plasma was quantifiable, FTC-TP was as well in 90.5% of the samples. The half-life of FTC-TP in DBS is short relative to that of TFV-diphosphate (TFV-DP), making it a surrogate for TFV-FTC detection in plasma. FTC-TP can be quantified in DBS simultaneously with TFV-DP, which quantifies cumulative adherence to TDF-FTC. (The clinical trials discussed in this article have been registered at ClinicalTrials.gov under identifiers NCT01040091, NCT02022657, NCT00458393, NCT01772823, and NCT02012621.)

Oral preexposure prophylaxis (PrEP) using coformulated tenofovir disoproxil fumarate and emtricitabine (TDF-FTC) has proven effective in preventing HIV infection in high-risk individuals (1–7). Unfortunately, PrEP efficacy has not been consistent across all studies, mostly due to variations in drug adherence (8–10). Multiple studies have demonstrated that sustained drug adherence and exposure are the main factors that determine success in PrEP (1, 5). However, despite its importance, no gold standard measure of antiretroviral adherence is currently available in routine clinical practice, and adequately quantifying adherence continues to be a challenge.

Plasma and intracellular tenofovir (TFV) and TFV-diphosphate (TFV-DP) levels have been shown to be powerful markers of adherence to PrEP (1, 5, 11). In particular, TFV-DP in red blood cells (RBCs), measured using dried blood spots (DBS), was found to be a strong marker of cumulative adherence to TDF-FTC and highly predictive of PrEP efficacy in men who have sex with men (MSM) (5, 7, 12). This is due to the uniquely long intracellular half-life (17 days) of TFV-DP in RBCs (and DBS), which leads to high accumulation with optimal adherence, so that adherence gradients can be estimated. This is informative about cumulative TDF dosing (adherence) over an extended period (6). However, because of its long half-life, TFV-DP in DBS is unable to discriminate between patterns of recent versus remote dosing and cannot adequately detect variations in very recent dosing.

Similar to TFV, FTC (the other component of the currently approved PrEP regimen) is also phosphorylated and trapped inside RBCs as FTC-triphosphate (FTC-TP) (13), with the advantage that it can be simultaneously quantified in DBS, along with TFV-DP. Although the pharmacokinetics of TFV-DP in DBS have been defined (6), our current knowledge about the disposition of FTC-TP in this matrix is limited. In addition, it remains unknown whether FTC-TP in DBS can provide adherence information complementary to that provided by TFV-DP.

In this study, we aimed to characterize the pharmacokinetics of FTC-TP in DBS and to evaluate its utility as a marker of recent dosing with TDF-FTC.

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MATERIALS AND METHODS

DBS samples from several studies were collected and used for data analyses; each study is briefly described below. The standard dose of 300 mg TDF-200 mg FTC was used in all studies. The local institutional review boards approved all the studies, and informed consent was obtained.

Cell-PrEP. Cell-PrEP (ClinicalTrials registration no. NCT01040091) was a prospective, observational, intensive pharmacokinetic study conducted at the University of Colorado-Anschutz Medical Campus in healthy HIV-negative and treatment-naive HIV-infected adult male and female participants aged 18 to 55 years. HIV-negative participants received daily coformulated TDF-FTC for 30 days, followed by 30 days off drug (the washout period), while HIV-infected participants received daily coformulated TDF-FTC plus 600 mg efavirenz and were followed for a total of 60 days. Whole blood for DBS was collected at 2 h postdose on days 1, 3, 7, and 20 and at 1, 2, 4, 8, and 24 h postdose on day 30 in HIV-negative and HIV-infected participants and randomly on days 35, 45, and 60 in the HIV-negative group. Participants fasted overnight prior to the dosing visits. Medication adherence was assessed by self-report and pill counting and with a dosing calendar.

DOT-DBS. DOT-DBS (ClinicalTrials registration no. NCT02022657) is an ongoing, prospective, randomized, observational, intensive pharmacokinetic study of directly observed oral coformulated TDF-FTC being conducted at the University of Colorado-Anschutz Medical Campus and the San Francisco Department of Public Health among healthy HIV-negative adult male and female participants aged 18 to 50 years. The participants were randomized to one of the following patterns of adherence to TDF-FTC: 100% (daily dosing), 67% (either 2 days on and 1 day off dosing or 2 weeks on and 1 week off dosing), or 33% (either 1 day on and 2 days off dosing or 1 week on and 2 weeks off dosing) for a total of 12 weeks, followed by a 12-week washout, and then by another randomly assigned 12-week dosing period with a different adherence pattern. All the dosing events in the study are witnessed either in person or via mobile videoconferencing. DBS are being collected at convenient times postdose (untimed) at 2, 4, 6, 8, 10, 12, 15, 18, 21, 24, 26, 28, 30, 32, 34, and 36 weeks after study initiation.

iPrEx-OLE. The iPrEx Open Label Extension (iPrEx-OLE) study (ClinicalTrials registration no. NCT00458393) was a 72-week-long multinational study that evaluated uptake of and adherence to PrEP in men and transgender women who have sex with men from three previous randomized controlled trials: Adolescent Medicine Trials Network for HIV/AIDS Interventions (ATN) 082, iPrEx, and the US Safety Study (5). Participants were males (gender at birth) older than 18 years who reported having had anal intercourse with men and had previously participated in a trial of daily oral PrEP with TDF-FTC (iPrEx and ATN 082) or TDF alone (U.S. Safety Study). Upon enrollment, all the participants were offered daily oral PrEP with TDF-FTC if they were HIV antibody negative and had no symptoms of acute HIV infection. Visits were done at enrollment and at weeks 4, 8, 12, 24, 36, 48, 60, and 72 after starting PrEP, and paired plasma-DBS samples were collected at a visit prior to week 24.

ATN 110. ATN 110 (ClinicalTrials registration no. NCT01772823) was a 48-week, multicenter, open-label study that combined PrEP with evidence-based behavioral risk reduction interventions and sexual health and adherence promotion counseling in the United States. Eligible participants were 18- to 22-year-old non-HIV-infected MSM who reported high-risk behavior for HIV infection in the preceding 6 months (12). Upon enrollment, all participants were offered daily oral PrEP with TDF-FTC if they were HIV antibody negative and had no symptoms of acute HIV infection. Study visits occurred at baseline, monthly through week 12, and quarterly through week 48. DBS and plasma samples were serially collected in a subset of participants.

13-2104. The 13-2104 study (ClinicalTrials registration no. NCT02012621) is an ongoing observational cohort study currently being conducted at the University of Colorado Hospital that is prospectively evaluating the relationship of TFV-DP and FTC-TP levels in DBS with virologic suppression in HIV-infected participants on TDF-FTC-based antiretroviral therapy. DBS (obtained from venipuncture and fingerstick) are being collected at the time of the patients' regular clinic visits within a 48-week period.

DBS processing and drug assays. After venipuncture, 25 μl of whole blood from EDTA tubes was spotted five times onto 903 Protein Saver cards (Whatman/GE Healthcare, Piscataway, NJ); allowed to dry for at least 2 h (up to overnight); and placed in plastic bags with humidity indicators, which were stored in a sample box with desiccant at −20°C or −80°C until analysis (14). For drug analysis of FTC-TP in DBS, a 3-mm-diameter disk was punched from the blood spot with a micropuncher. The punched disk was placed in a microcentrifuge tube with 500 μl of 70:30 methanol-H_2O and sonicated for 10 min, constituting a lysed cell matrix that was stored at −80°C until analysis using liquid chromatography-tandem mass spectrometry (LC–MS-MS), which has been previously validated with an intra- and interextraction coefficient of variation of ≤12.9% and an intercard percent difference of ±12.4% (6, 14, 15). Plasma TFV and FTC were measured using a previously validated LC–MS-MS method (16). The lower limit of quantification (LLOQ) for FTC-TP in DBS was 0.1 pmol/sample; the LLOQ for both TFV and FTC in plasma was 10 ng/ml.

Pharmacokinetic analysis of FTC-TP in DBS. The half-life and steady-state concentration of FTC-TP were estimated using one-compartment, first-order pharmacokinetics. In the accumulation phase, the individual average steady-state concentration (C_{ssavg}) for each participant was estimated by fitting a monoexponential equation to the concentration-time data from first dose to steady state: $C_t = C_{ssavg} \times (1 - \exp^{-kt})$, where C_t is the concentration at a given time on therapy, t is time on therapy, C_{ssavg} is the fitted steady-state concentration, and k is the fitted elimination rate constant. In the elimination phase, declining data points from the last day of dosing in individual HIV-negative participants (day 30) were analyzed to determine the elimination rate constants (k_e) of FTC-TP in DBS by fitting a linear regression to the natural log-transformed concentrations obtained at 1, 2, 4, 8, and 24 h postdose using GraphPad (version 6.00 for Windows; GraphPad Software, La Jolla, CA). FTC-TP levels in DBS that were below the limit of quantification (BLQ) in the accumulation phase were assigned an imputed value of 0.05 pmol/punch, which is halfway between zero and the LLOQ of the assay. The difference in the accumulation half-life between HIV-negative and HIV-infected individuals was evaluated using an unpaired t test, and a P value of <0.05 was considered to be statistically significant. Data are presented as means and 95% confidence intervals (CI) unless otherwise indicated.

Postdose detectability of FTC-TP in DBS. The quantification of FTC-TP in DBS in relation to the time postdose was evaluated utilizing samples obtained from HIV-negative participants enrolled in DOT-DBS. In this analysis, the presence or absence of FTC-TP was assessed in DBS obtained at >0 to 12, >12 to 24, >24 to 36, >36 to 48, >48 to 60, >60 to 72, >72 to 84, >84 to 96, and >96 h post-TDF-FTC dose. FTC-TP in DBS was defined as quantifiable or BLQ, and the proportions of DBS samples with quantifiable FTC-TP within each time frame and the time to nondetectability after TDF-FTC dosing were determined.

Agreement between TFV-FTC in plasma and FTC-TP in DBS. To evaluate the concordance in drug detectability between TFV-FTC in plasma and FTC-TP in DBS, the presence or absence of quantifiable drug in paired plasma-DBS samples obtained at various study visits in iPrEx-OLE and ATN 110 was assessed. To do this, the agreement of a BLQ level of both TFV and FTC in plasma with a BLQ level of FTC-TP in DBS and of the quantification of either TFV or FTC in plasma with the quantification of FTC-TP in DBS was determined. The difference between the proportions of quantifiable TFV-FTC in plasma and quantifiable FTC-TP in DBS was assessed using McNemar's test for paired nominal data. A P value of <0.05 was considered to be statistically significant.

FTC-TP in DBS obtained via venipuncture versus fingerstick. The correlation between FTC-TP levels in DBS obtained from peripheral blood via venipuncture (25 μl of blood spotted onto 903 Protein Saver Cards) versus DBS obtained from capillary blood via fingerstick was eval-

TABLE 1 Demographic characteristics of participants per study

Study (*n*)	Median age (range) (yr)	Gender (no.)		Race/ethnicity (no.)				HIV status (no.)	
				Not Hispanic			Hispanic		
		Male	Female	White	Black/African American	Asian/mixed/other		Negative	Infected
Cell-PrEP (23)[a]	31 (20–52)	16	7	9	11 (4 females)	0	3	13	10
DOT-DBS (29)[b]	29 (21–50)	12	17	16	6 (3 females)	1	6	29	0
iPrEx-OLE (337)[c]	29 (19–70)	337	0	59	40	43	195	337	0
ATN 110 (108)[c]	20 (18–22)	108	0	29	53	3	23	108	0
13-2104 (30)[d]	49 (26–63)	27	3	20	2 (1 female)	3	5	0	30

[a] Data for the accumulation phase analysis were available for 23 Cell-PrEP participants (including HIV negative and HIV infected); day 30 data for the terminal half-life analysis were available for 7 Cell-PrEP participants (all HIV negative).
[b] Including participants who had available data at the time of analysis.
[c] Including participants who had paired plasma and dried blood spot samples at the time of the study visit.
[d] Convenience sample obtained at the time of the study visit.

uated in HIV-negative participants enrolled in DOT-DBS and in HIV-infected participants enrolled in the 13-2104 study. The rationale was that, during assay validation, it was found that FTC in plasma can be converted to FTC-TP in RBCs as whole blood sits at room temperature prior to spotting when the blood is collected by venipuncture (14). This improves the utility of detecting a recent PrEP dose, but it suggests that fingerstick DBS (which is fixed immediately) may show different results. Briefly, a lancet puncture was performed after sterile cleaning of the skin of the index, middle, or annular finger distal phalanx. After the puncture, the first drop of blood was discarded, and subsequent blood drops were collected on 903 Protein Saver Cards, which were processed and stored as described above (6, 14). The correlation between venipuncture and fingerstick FTC-TP levels was evaluated using a Pearson correlation.

Preappointment dosing. The potential clinical application of FTC-TP in DBS as a marker of recent dosing was evaluated by its ability to detect "white-coat" adherence in individuals who appeared to be nonadherent by their levels of TFV-DP but who took a dose of TDF-FTC prior to their study visits. To accomplish this, the proportions of DBS samples obtained from participants enrolled in iPrEx-OLE and ATN 110 in which FTC-TP was quantifiable were determined according to the following cumulative-adherence dosing categories based on the levels of TFV-DP (5): <2 doses per week (<350 fmol/punch or BLQ), 2 or 3 doses per week (350 to 699 fmol/punch), and ≥4 doses per week (≥700 fmol/punch).

RESULTS

Study population. The demographic characteristics of the participants enrolled in Cell-PrEP, DOT-DBS, iPrEx-OLE, ATN 110, and 13-2104 are shown in Table 1.

Pharmacokinetics of FTC-TP in DBS–Cell-PrEP. Following the first dose, FTC-TP reached a maximal concentration (C_{max}) at a median (range) of 4 (2 to 8) hours. In the accumulation phase, all the data points from 13 HIV-negative and 10 HIV-infected Cell-PrEP participants were included in the analysis, which yielded a mean half-life of 38 (29 to 55) hours and a C_{ssavg} of 0.26 (0.24 to 0.29) pmol/punch (Fig. 1a). No difference in half-life was identified between HIV-negative and HIV-infected participants ($P = 0.43$). Declining data points were available in 7 HIV-negative Cell-PrEP participants and were included in the elimination phase analysis. The mean elimination half-life of FTC-TP in DBS was 35 (23 to 47) hours (Fig. 1b). During the washout phase, FTC-TP concentrations in DBS were BLQ in all except 1 participant at day 35 (5 days after the last dose) and in all participants at days 45 and 60, whereas TFV-DP was well within the quantifiable range at all these time points (data not shown).

Detection of FTC-TP in DBS after TDF-FTC dosing (DOT-DBS). A total of 256 DBS samples post-TDF-FTC dosing were obtained from 29 participants enrolled in the DOT-DBS study and included in the analysis. The proportion of samples with quantifiable FTC-TP in each postdose category is shown in Table 2. All BLQ samples ($n = 13$) obtained within the 0- to 12-h post-TDF-FTC dose period were obtained within 30 min after the dosing episode and occurred in participants who were not randomized to 100% adherence (4 were randomized to 67%, and 3 were

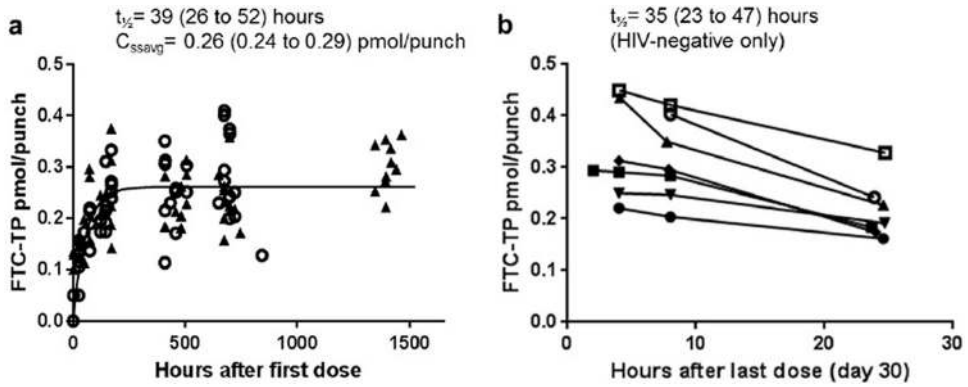

FIG 1 Mean (95% CI) accumulation phase (a) and terminal phase (b) half-lives ($t_{1/2}$) of FTC-TP in DBS in HIV-negative and HIV-infected participants enrolled in Cell-PrEP (each symbol represent a different participant). In the accumulation phase (a), the circles indicate HIV-negative individuals and the triangles indicate HIV-infected individuals. Solid line represents the fitted FTC-TP concentration.

TABLE 2 Proportions of DBS samples with detectable FTC-TP levels according to time post-TDF-FTC dose

Sample parameter	Value at time (h) postdose								
	>0–12	>12–24	>24–36	>36–48	>48–60	>60–72	>72–84	>84–96	>96
Total no.	76	38	36	13	2	1	2	1	87
No. detectable	63	38	35	7	1	0	1	0	0
No. BLQ	13[a]	0	1	6	1	1	1	1	87
% detectable	83	100	97	54	50	0	50	0	0

[a] Twelve out of 13 occurred within 30 min of dosing following a prolonged drug holiday (≥72 h).

randomized to 33%); 12 of the samples were obtained from individuals who had been off drug for at least 72 h. Collectively, 143 out of 163 (88%) samples obtained between 0 and 48 h post-TDF-FTC dose had quantifiable FTC-TP levels compared to only 2 out of 93 (2%) samples obtained >48 h postdose. None of the 87 samples obtained >96 h postdose had detectable FTC-TP in DBS.

Concordance of TFV-FTC in plasma with FTC-TP in DBS (iPrEx-OLE and ATN 110). A total of 746 paired plasma-DBS samples obtained from 445 patients enrolled in iPrEx-OLE and ATN 110 were analyzed (Table 1). The concordance of TFV and FTC in plasma with FTC-TP in DBS at both ends of the quantification continuum was determined. When both TFV and FTC were BLQ in plasma, FTC-TP was BLQ in DBS 98.9% of the time (Table 3). In comparison, when either TFV or FTC was quantifiable in plasma, FTC-TP was quantifiable in DBS 90.5% of the time (Table 3). Overall, when TFV/FTC in plasma and FTC-TP in DBS were discordant, there was a higher probability that this discordance was due to a quantifiable TFV/FTC level in plasma when FTC-TP in DBS was BLQ ($P < 0.0001$).

Correlation between FTC-TP in DBS obtained via venipuncture versus fingerstick (13-2104 and DOT-DBS). A total of 51 paired DBS samples (30 from HIV-infected individuals) obtained at the same visit via venipuncture and fingerstick were analyzed. The mean time from venipuncture to DBS spotting was 3.9 (3.3 to 4.3) hours. Of these samples, 9 (17.6%) obtained via venipuncture versus 11 (21.6%) obtained via fingerstick (including all 9 samples obtained via venipuncture) were BLQ. FTC-TP concentrations in venipuncture and fingerstick samples were strongly correlated ($r = 0.93$; $P < 0.0001$).

Preappointment dosing (iPrEx-OLE and ATN 110). Only one iPrEx-OLE participant who had TFV-DP BLQ had quantifiable FTC-TP in DBS, in comparison with none of the ATN 110 participants. Figure 2 depicts the proportion of quantifiable FTC-TP in DBS according to TDF-FTC dosing categories. In the category of <2 doses per week, 34% (57/166) of iPrEx-OLE and 32% (16/50) of ATN 110 participants who had low, but quantifiable, TFV-DP levels showed evidence of preappointment dosing (i.e., quantifiable FTC-TP at the time of the study visit). In the category of 2 or 3 doses per week, 79% (68/86) of iPrEx-OLE and 75% (45/60) of ATN 110 participants had quantifiable FTC-TP in DBS, consistent with recent dosing prior to the study visit. In comparison, 96% (255/267) of iPrEx-OLE and ATN 110 participants (combined) in the category of 4 or more doses per week had evidence of quantifiable FTC-TP in DBS. Thus, only 4% (6/155) of iPrEx-OLE and 5% (6/112) of ATN 110 participants had evidence of recent drug discontinuation (i.e., FTC-TP BLQ) when taking 4 or more TDF-FTC doses per week.

TABLE 3 Concordance of TFV-FTC in plasma versus FTC-TP in DBS in paired plasma-DBS samples from iPrEx-OLE and ATN 110

Drug	No. (%) positive ($n = 746$)		
	FTC-TP quant	FTC-TP BLQ	Total
TFV or FTC quant[a]	439 (90.5)	46 (9.5)	485
TFV-FTC BLQ[b]	3 (1.1)	258 (98.9)	261

[a] A total of 485 samples had detectable TFV or FTC in plasma.
[b] A total of 261 samples had both TFV and FTC BLQ in plasma; quant, quantifiable.

DISCUSSION

In this study, we describe the pharmacokinetics of FTC-TP in DBS and identify a half-life of approximately 1.5 days in this matrix. This is significantly shorter than the 17-day half-life of TFV-DP, the other component of the only currently FDA-approved PrEP medication (6). These FTC-TP findings are consistent with its disposition in peripheral blood mononuclear cells, in which FTC-TP was found to have a terminal half-life of 37 to 39 h (17, 18) and an anticipated time to steady state of approximately 3 to 7 days (19, 20). The expected FTC-TP C_{ssavg} of approximately 0.26 pmol/punch is just moderately above the LLOQ of the assay (0.1 pmol/punch), so that concentrations fall BLQ at about the same time that plasma TFV (half-life, 14 h) and FTC (half-life, 10 h) levels fall below 10 ng/ml (13). These pharmacokinetic characteristics are well suited to the utilization of FTC-TP in DBS as a marker of recent dosing with TDF-FTC in PrEP and HIV treatment. To further evaluate its utility, we showed that the quantification of FTC-TP in DBS has excellent concordance with the quantification of TFV-FTC in plasma (with a LLOQ of 10 ng/ml) and that it detects a TDF-FTC dosing episode that occurred within

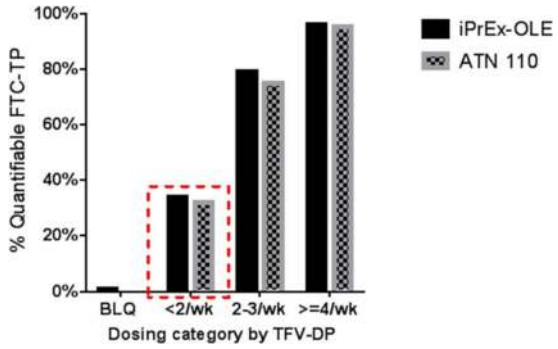

FIG 2 Proportions of samples with quantifiable FTC-TP in 746 DBS samples obtained from 445 PrEP participants enrolled in iPrEx-OLE and ATN 110 according to the dosing category (determined by TFV-DP in DBS). The dashed red square represents individuals with evidence of preappointment dosing. <2 doses per week, TFV-DP BLQ to <350 fmol/punch; 2 or 3 doses per week, TFV-DP 350 to 699 fmol/punch; ≥4 doses per week, TFV-DP ≥700 fmol/punch.

the previous 36 to 48 h, but not beyond 96 h postdose. This is similar to the "look back" periods reported for both TFV and FTC in plasma, with a LLOQ of 10 ng/ml (13), and supports the use of FTC-TP in DBS as a surrogate for plasma for recent dosing.

FTC-TP was readily detectable in DBS due to the large number of RBCs (~12 million) that are contained in a 3-mm DBS punch (6). An advantage to measuring FTC-TP in DBS is that it can be quantified simultaneously with TFV-DP in the same sample analysis. In addition, FTC-TP in DBS shows a strong correlation between venipuncture and fingerstick, which allows potential self-collection and its use in the field and in resource-limited settings. Collectively, these characteristics make FTC-TP in DBS a useful biomarker to objectively measure recent adherence to TDF-FTC.

Given the difficulties regarding the accurate quantification of antiretroviral adherence for treatment and prevention, new objective measures of drug exposure and adherence that can discriminate adherence variations throughout treatment are needed. For example, although a TFV-DP level of ≥700 fmol/punch in DBS (consistent with taking ≥4 TDF-FTC doses per week) has been associated with high PrEP efficacy, it provides limited data regarding recent dosing patterns (6). In this context, the inclusion of FTC-TP provides recent dosing information that could be incorporated into future adherence-response studies and clinical practice. When combined, these two pharmacological measures constitute a more comprehensive objective quantification of TDF-FTC intake over the preceding 1 to 2 months (TFV-DP) and 36 to 48 h (FTC-TP).

The potential clinical application of FTC-TP in DBS was demonstrated by the detection of recent TDF-FTC dosing in PrEP study participants with variable degrees of cumulative adherence. The high proportion (32 to 34%) of quantifiable FTC-TP observed in the group taking <2 TDF-FTC doses per week with low, but detectable, TFV-DP levels is consistent with low adherence with an intention to improve adherence in proximity to a study visit, which could be used as an opportunity to study predictors of this behavior in future trials and clinical practice. In contrast, the group of individuals in whom both TFV-DP and FTC-TP were BLQ indicates nearly complete nonadherence, with virtually no study drug intake over approximately the preceding month.

In terms of limitations, this study was based on the utilization of FTC-TP in DBS as a qualitative (yes/no) measure of recent TFV-FTC dosing and did not evaluate it as a quantitative measure. When using blood from venipuncture, the level of FTC-TP in RBCs is influenced by high levels of FTC in plasma (14). While this does not impair the utility of detecting a recent dose, it does prevent the use of the actual FTC-TP value in modeling studies. A potential strategy to overcome this limitation is collection of DBS using fingerstick, since the capillary blood obtained by this process is immediately fixed in the DBS and would not allow further FTC uptake and phosphorylation by the RBCs. The strong correlation ($r = 0.93$) between FTC-TP in DBS collected via venipuncture versus fingerstick suggests that either approach could be used in the field. Another limitation was that FTC-TP in DBS was BLQ in a fraction of cases when dosing was within 30 min of the blood draw after a recent period of at least 72 h off drug. This suggests that FTC-TP in DBS may not pick up a prolonged drug holiday followed by white-coat dosing within 30 min of a clinic visit (although TFV-DP would show the prolonged holiday). More work is needed to understand how to use drug concentrations to infer patterns of dosing.

In conclusion, FTC-TP exhibits pharmacokinetics distinctive from those of TFV-DP in DBS, which supports its use as a measure of recent dosing with TDF-FTC and as a surrogate for plasma TFV/FTC levels at an assay LLOQ of 10 ng/ml. Future studies are needed to examine the clinical utility of FTC-TP in combination with TFV-DP as a measure of adherence to HIV PrEP and treatment.

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