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Steady-state pharmacokinetics of plasma tenofovir alafenamide (TAF), tenofovir (TFV) and emtricitabine (FTC), and intracellular TFV-diphosphate and FTC-triphosphate in HIV-1 infected old Japanese patients treated with bictegravir/FTC/TAF

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Abstract: Emtricitabine (FTC) plus tenofovir alafenamide (TAF) has demonstrated efficacy and safety for pre-exposure prophylaxis (PrEP) to prevent HIV-1 infection. We measured the plasma PK of FTC, tenofovir (TFV), and TAF in a steady-state pharmacokinetic (PK) study of bictegravir/FTC/TAF in HIV-1-infected patients. Furthermore, validated liquid chromatography-tandem mass spectrometry was used to measure intracellular TFV-diphosphate (DP) and FTC-triphosphate (TP), the active metabolites of TFV and FTC, respectively. Plasma and dried blood spot samples were collected from 10 male patients aged ≥ 50 years at various time intervals: 0 (trough), 1, 2, 3, 4, 6, 8, 12, and 24 h after drug administration. The mean ± standard deviation of plasma PK parameters were as follows: The maximum concentrations of TAF, TFV, and FTC were 104.0 ± 72.5, 27.9 ± 5.2, and 3,976.0 ± 683.6 ng/mL, respectively. Additionally, their terminal elimination half-lives were 0.6 ± 0.5, 31.6 ± 10.4, and 6.9 ± 1.4 h, respectively. These results were consistent with previously reported data. The intracellular levels of TFV-DP and FTC-TP varied widely among individuals; however, they remained stable over 24 h in each individual at approximately 1,000–1,500 and 2,000–3,000 fmol/punch, respectively, indicating that plasma concentrations did not affect the intracellular concentrations of their active metabolites. These results demonstrated that measuring intracellular TFV-DP and FTC-TP could be useful for monitoring adherence to PrEP in clients on this regimen.

Keywords: pre-exposure prophylaxis, plasma concentration, intracellular concentration, liquid chromatography-tandem mass spectrometry, dried blood spot

Introduction

Bictegravir/emtricitabine/tenofovir alafenamide (BIC/FTC/TAF) is well known as an effective and well-tolerated regimen for the initial treatment of HIV-1 infection in adults (*1*). This medication consists of an integrase strand transfer inhibitor (INSTI) of BIC and two nucleoside reverse transcriptase inhibitors (NRTIs), FTC and TAF (*2*). However, the pharmacokinetic (PK) data for the elderly Japanese population are currently limited. Here, we conducted a prospective steady-state PK study of BIC/FTC/TAF in elderly Japanese patients using previously published BIC data (*3*). In this study, we examined the remaining PK data for plasma FTC, TAF, and tenofovir (TFV), an intermediate metabolite of TAF. In addition, we examined the intracellular concentrations of FTC-triphosphate (TP) and TFV-diphosphate (DP), the active metabolites of FTC and TFV, respectively.

To prevent new cases of HIV infections, recent guidelines recommend using oral tenofovir disoproxil fumarate (TDF) or TAF and FTC as pre-exposure prophylaxis (PrEP) for men who have sex with men (MSM) to effectively reduce the likelihood of acquiring HIV, using either daily or event-controlled regimens (*4-7*). Since the viral load is used as an objective indicator to evaluate HIV treatment, the lack of a load that can be used in daily practice for PrEP makes it difficult to monitor adherence accurately. Non-pharmacological methods such as self-reporting, pill counts, and refill records have been used as alternative measures to determine adherence to PrEP (*8,9*). Recently, several pharmacological methods have been developed

to measure drug concentrations. TFV and FTC concentrations in the plasma, urine, and saliva have been used as objective indicators of adherence. Urine, saliva, and plasma samples are easily collected and can be used to confirm recent drug intake. However, given the short half-lives of these samples, drug concentrations must be examined soon after the drug administration, even though PrEP typically requires drugs to be administered in the evening. Urine and saliva concentrations vary significantly (*10-13*). Intracellular TFV-DP in peripheral blood mononuclear cells (PBMCs), the active ingredient in both TDFs and TAFs, is the most appropriate choice for assessing efficacy. Although TFV-DP has persistently high concentrations in PBMCs, the processing of PBMCs is time-consuming and requires accurate cell counts; therefore, it is not feasible on a large scale (*14*). To address these limitations, TFV-DP has been developed and validated in red blood cells (RBCs) using dried blood spots (DBS) and is being used as an adherence biomarker in several laboratories (*15-17*). However, methods for the measurement of intracellular TFV-DP and FTC-TP using DBS have not yet been established in Japanese participants.

In this study, we first examined the PK data of FTC, TAF, and TFV in plasma obtained from a prospective steady-state PK study of BIC/FTC/TAF in elderly HIV-1-infected Japanese patients (*3*). Furthermore, we prepared samples of DBS at the same time points as the plasma samples and developed a method to accurately measure the concentrations of intracellular TFV-DP and FTC-TP in DBS. We subsequently investigated the most appropriate markers for monitoring adherence to PrEP.

Materials and Methods

Subjects

This prospective cohort study was conducted at AIDS Clinical Center of National Center for Global Health and Medicine. Male HIV-1-infected Japanese patients with suppressed HIV RNA levels and without BIC-containing antiretroviral regimens were recruited (Supplemental files, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=67*). Peripheral blood samples were collected from each participant using heparin tubes for plasma and DBS analyses at multiple time points: 0 (trough), 1, 2, 3, 4, 6, 8, 12, and 24 h after drug administration (*3*). This research was conducted in compliance with the Declaration of Helsinki and national and institutional standards. The Institutional Review Board for Clinical Research of National Centre for Global Health and Medicine approved the study protocol (approval no. NCGM-G-003461-00), and all participants provided written informed consent prior to enrollment. The study protocol was registered at UMIN-CTR (UMIN00004113).

Measurement of plasma tenofovir alafenamide, tenofovir, and emtricitabine concentrations

TAF, TFV, FTC, TAF-d5 (internal standard [IS] of TAF), TFV-d6 (IS of TFV), and FTC-^{13}C,$^{15}N_2$ (IS of FTC) were purchased from Toronto Research Chemicals (Toronto, ON, Canada). Blank human plasma samples were purchased from Cosmo Bio (Tokyo, Japan). Plasma samples (100 μL) were deproteinized with ethanol. An ACQUITY UPLC H-Class system (Waters, Milford, MA, USA) and QTRAP 6500 mass spectrometer (AB Sciex, Framingham, MA, USA) equipped with an electrospray ionization source were used to measure the plasma concentrations of TAF, TFV, and FTC. Chromatographic separation was achieved on an ACQUITY UPLC HSS T3 column (50 × 2.1 mm, particle size 1.8 μm, Waters) using gradient elution with mobile phases A (20 mmol/L ammonium acetate containing 0.1% formic acid) and B (20 mmol/L ammonium acetate containing 0.1% formic acid in methanol/water (9:1, v/v)) at a flow rate of 0.4 mL/min. The gradient method consisted of the percentage change in mobile phase B in relation to time (0–0.5 min: 5% B; 0.5–2.7 min: 5 to 25% B; 2.7–4.5 min: 25 to 75% B; 4.5–6.0 min: 75 to 100% B; 6.0–|7.0: 100% B; 7.0–10.0 min: 100 to 5% B). The injection volume was 5 μL, and the run time was 10 min. The mass spectrometer was operated in the positive electrospray ionization mode. The mass transitions were m/z 477.3→364.1 for TAF and m/z 482.3→369.1 for TAF-d5, m/z 288.1→176.2 for TFV and m/z 294.1→182.2 for TFV-d6, and m/z 248.2→130.1 for FTC and m/z 251.2→133.1 for FTC-^{13}C,$^{15}N_2$. The calibration curves for TAF, TFV, and FTC were linear within the range of 0.5–500 ng/mL (r2 = 0.99). The intra- and inter-day precision and accuracy of TAF, TFV, and FTC in plasma had a coefficient of variation (CV) of 15.0%. The PK parameters were determined by non-compartmental analysis using the Phoenix WinNonlin version 8.2 software (Certara, Princeton, NJ, USA).

Measurement of intracellular tenofovir diphosphate and emtricitabine triphosphate concentrations

TFV-DP (tetra-ammonium salt) and FTC-TP (tetra-ammonium salt) were purchased from Toronto Research Chemicals. Meanwhile, $^{13}C_5$-TFV-DP (the IS of TFV-DP and FTC-TP) was purchased from Moravek Biochemicals (Brea, CA, USA).

We extracted a total of five punches (3 mm each) from the Whatman Protein Saver card of each patient in a microcentrifuge tube with 25 μL of methanol/water (1:1, v/v) and 400 μL of IS solution. Subsequently, 400 μL of supernatant from each tube was purified *via* a Solid Phase Extraction 96-well plate (Waters). A Shimadzu liquid chromatography system coupled with an 8050 triple quadrupole mass spectrometer (Kyoto, Japan) was then used to measure intracellular concentrations of the

analytes of interest. Analyte separation was achieved on an Atlantis Premier BEH C18 AX Column (50 × 2.1 mm, particle size 1.7 μm, Waters) at 40 °C with a 5.5-min gradient setting and 0.6 mL/min flow rate, using mobile phase A: 0.2% acetic acid/ methanol (1:4, v/v) and mobile phase B: (1M ammonia solution/1M ammonium acetate/water = 1:0.01:200, v/v)/methanol (9:1, v/v).

The gradient method consisted of the percentage change in mobile phase B over time (0–0.5 min: 0% B; 0.5–2.0 min: 0 to 95% B; 2.0–4.0 min: 95 to 100% B; 4.0–5.0 min: 100 to 0% B; 5.0–5.5 min: 0% B). The injection volume was 7 μL, and the run time was 5.5 min per sample. A mass spectrometer was used in positive electrospray ionization mode to detect the following analytes: m/z 448→176 for TFV-DP, m/z 488→130 for FTC-TP, and m/z 453→275 for $^{13}C_5$-TFV-DP. The quantifiable linear range for TFV-DP was 279–27901 fmol/punches ($r^2 = 0.99$), and that for FTC-TP was 256–25614 fmol/punches ($r^2 = 0.99$). The intra- and inter-day precision and accuracy of TFV-DP and FTC-TP were both within 15.0%.

Statistical analysis

Plasma PK parameters are expressed as mean ± standard deviation (SD). Plasma concentrations of TAF, TFV, and FTC and intracellular TFV-DP and FTC-TP at each time point are presented as the median ± interquartile range. Simple linear regression was used with *p* values < 0.05, indicating the presence of a statistically significant difference. All statistical analyses were performed using SPSS Statistics software version 23 (IBM, Armonk, NY, USA).

Results

Plasma concentrations of TAF, TFV, and FTC

Plasma samples were collected from the ten enrolled participants at the nine aforementioned time points before being measured (Table 1 and Figure 1). The maximum concentrations (C_{max}, mean ± SD) of TAF, TFV, and FTC were 104.0 ± 72.5 ng/mL (at 1.0 ± 0.0 h after dosing), 27.9 ± 5.2 ng/mL (at 2.2 ± 2.1 h after dosing), and 3,976. 0 ± 683.6 ng/mL (at 1.3 ± 0.7 h after dosing), respectively. The trough concentrations (C_{trough}, mean ± SD) of TAF and TFV were less than 0.5 ng/mL and 15.8 ± 3.0 ng/mL, respectively, whereas for FTC they varied within the range of 181.8 ± 49.7 ng/mL. The areas under the concentration-time curves for the last 24-h dosing intervals ($AUC_{0–24}$, mean ± SD) of TAF, TFV, and FTC were 127.9 ± 82.6 h*mg/mL, 484. 8 ± 79.4 h*mg/mL, and 22,417.1 ± 6,168.4 h*mg/mL, respectively. Furthermore, the elimination terminal half-lives ($T_{1/2}$, mean ± SD) of TAF and FTC were 0.6 ± 0.5 h and 6.9 ± 1.4 h, respectively. It should be noted that $T_{1/2}$ of TFV was 31.6 ± 10.4 h, or it could be expressed as stable.

Intracellular concentrations of TFV-DP and FTC-TP in DBS

The intracellular concentrations of TFV-DP and TFV-TP varied widely among the 10 patients while remaining stable in each individual patient. The median ± interquartile ranges at each time point of 0, 1, 2, 3, 4, 6, 8, 12, and 24 h are summarized in Table 2. In summary, the intracellular concentrations were approximately 1,000–1,500 fmol/punch for TFV-DP and 2,000–3,000 fmol/punches for FTC-TP over 24 h (Figure 2).

Correlation between plasma and intracellular concentration

There was no correlation between the plasma and intracellular concentrations of the following pairs: TAF and TFV-DP, TFV and TFV-DP, and FTC and FTC-TP. This strongly indicated that plasma concentrations did not affect the intracellular active metabolites (Figure 3).

Discussion

We examined the PK parameters of TAF, TFV, and FTC in plasma and intracellular TFV-DP and FTC-TP in DBS at nine time points in elderly HIV-1-infected Japanese patients to determine the markers that were the most valuable objective indicators for monitoring adherence to PrEP with FTC/TAF.

Our method showed that all results were well within the acceptance criteria for method validation. Several assays have been previously developed to detect the intracellular concentrations of TFV-DP and FTC-

Table 1. Pharmacokinetic parameters of tenofovir alafenamide, tenofovir, and emtricitabine in plasma

Items	Tenofovir alafenamide (mean ± SD)	Tenofovir (mean ± SD)	Emtricitabine (mean ± SD)
C_{max} (ng/mL)	104.0 ± 72.5	27.9 ± 5.2	3,976.0 ± 683.6
T_{max} (h)	1.0 ± 0.0	2.2 ± 2.1	1.3 ± 0.7
C_{trough} (ng/mL)	< 0.5	15.8 ± 3.0	181.8 ± 49.7
AUC_{0-24} (h*ng/mL)	127.9 ± 82.6	484.8 ± 79.4	22,417.1 ± 6,168.4
$T_{1/2}$ (h)	0.6 ± 0.5	31.6 ± 10.4	6.9 ± 1.4

C_{max}, maximum plasma concentration; T_{max}, time to maximum plasma concentration; C_{trough}, trough plasma concentration; AUC, area under the plasma concentration-time curve; $T_{1/2}$, elimination half-life.

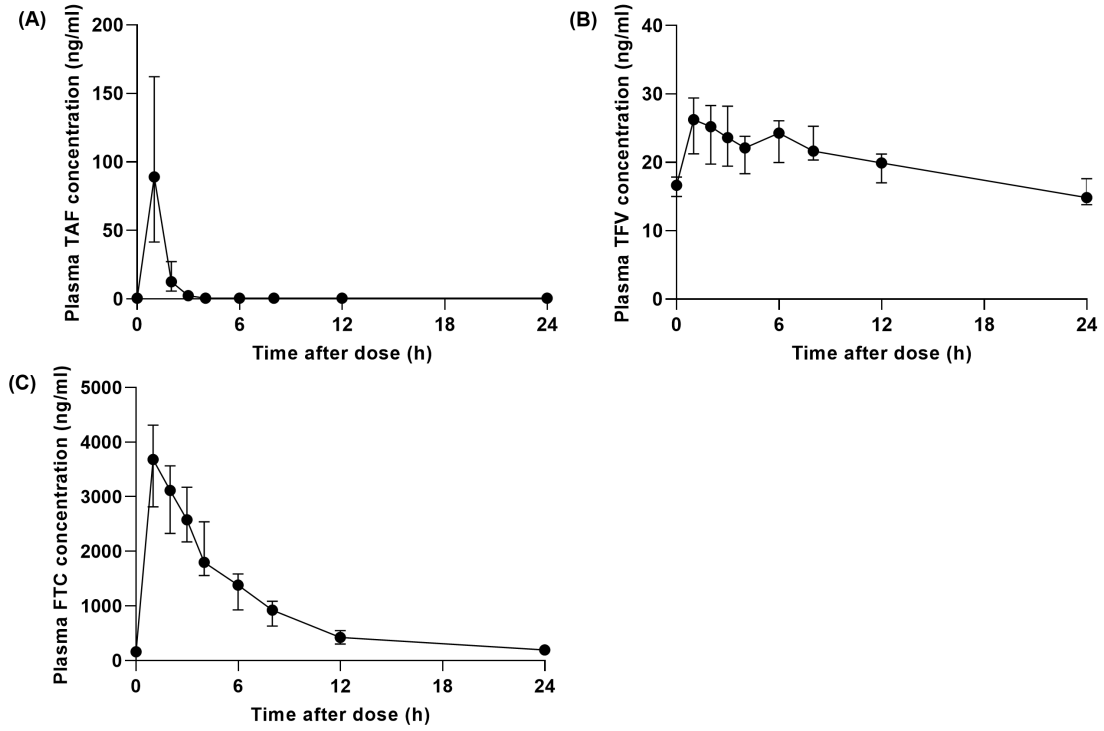

Figure 1. Pharmacokinetics of tenofovir alafenamide, tenofovir, and emtricitabine in plasma. (A) tenofovir alafenamide (TAF), **(B)** tenofovir (TFV), **(C)** emtricitabine (FTC). The data are illustrated as the median ± interquartile range.

Table 2. Pharmacokinetics of intracellular tenofovir-diphosphate and emtricitabine-triphosphate

Time after doses (hours)	0	1	2	3	4	6	8	12	24
TFV-DP (fmol/ punches)									
Median	1,214	1,161	1,335	1,086	1,354	1,113	1,373	1,191	1,597
75% Percentile	1,540	1,645	1,673	1,963	1,949	2,170	2,372	2,489	2,584
25% Percentile	1,066	782.9	966	837.6	965.4	910.7	1,002	802.5	830.9
FTC-TP (fmol/ punches)									
Median	1,550	2,316	1,818	2,166	2,539	1,725	2,657	1,527	1,640
75% Percentile	2,455	3,441	3,352	3,886	4,060	5,026	4,412	4,497	4,276
25% Percentile	836.1	1,027	1,451	1,153	936	1,069	972.3	977.5	607.6

TP using DBS (*15,18*); however to our knowledge (*19*), this is the first study to compare plasma and intracellular concentrations of the analytes of interest and their corresponding metabolites among Japanese patients taking BIC/FTC/TAF. Our results subsequently confirmed that the half-lives of intracellular TFV-DP and FTC-TP were significantly long, whereas their concentrations were stable and independent of the plasma concentrations of TAF, TFV, and FTC.

The plasma $T_{1/2}$ of TFV was long (31.6 h) or almost stable after FTC/TAF. The reason behind the long TFV $T_{1/2}$ could be explained as follows (*20*): After TAF is absorbed in plasma, it directly enters target cells, is subjected to ester hydrolysis by cathepsin A to TFV, and is subsequently phosphorylated to TFV-DP or slowly released from the cells into the plasma. Therefore, TFV has a low C_{max}, whereas its $T_{1/2}$ is stable. Therefore, plasma TFV concentrations could be used to monitor PrEP adherence, especially among PrEP users taking TFV/TAF. However, further studies on patients receiving FTC/TDF are necessary.

The efficacy of PrEP has been reported to be excellent in many previous studies and is recommended by several guidelines (*6,7*), provided that good adherence to treatment is maintained. Nonetheless, unlike HIV-1 treatment, which is currently based on a once-daily therapy strategy and where treatment success can be objectively assessed by plasma viral load, plasma drug concentrations among PrEP users would only be useful as markers if the dosing occurred on the same day. This might not be the case because they often take the medication before sexual intercourse, which might happen the day or night before.

According to our data, the plasma concentration of each drug did not correlate with the intracellular pharmacologically active metabolites. Since these intracellular metabolites have a significant longer duration, particularly after TDF/FTC or FTC/TAF dosing, TFV-DP remains in DBS for approximately 2 weeks. This results in a 25-fold accumulation from the

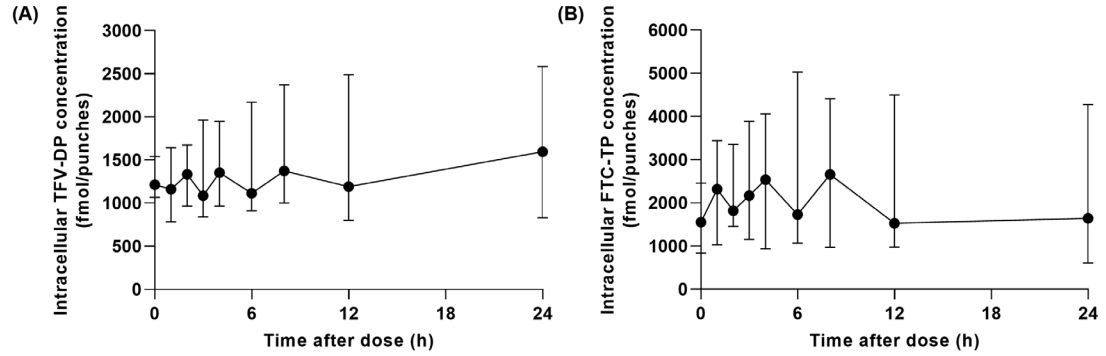

Figure 2. Pharmacokinetics of intracellular tenofovir-diphosphate and emtricitabine-triphosphate. (A) tenofovir-diphosphate (TFV-DP), **(B)** emtricitabine-triphosphate (FTC-TP). The data are illustrated as the median ± interquartile range.

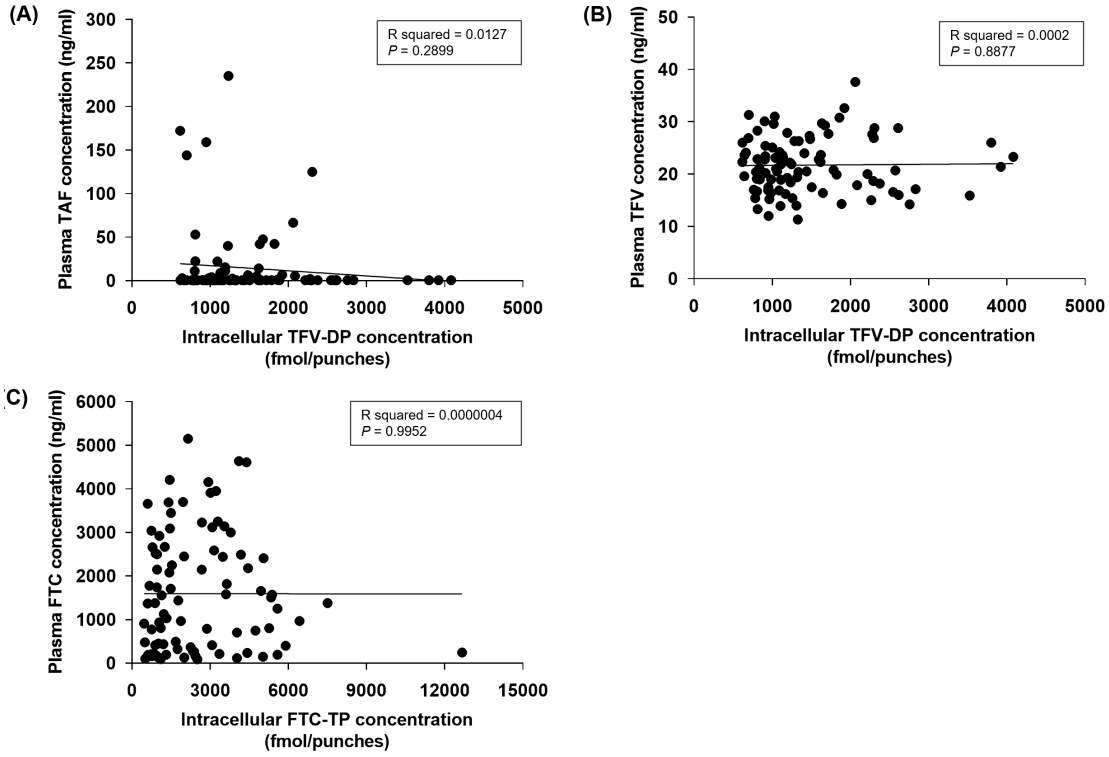

Figure 3. Correlations between plasma concentrations of tenofovir alafenamide, tenofovir, and emtricitabine and their corresponding intracellular drug concentrations. (A) tenofovir alafenamide (TAF) and tenofovir-diphosphate (TFV-DP), **(B)** tenofovir (TFV) and tenofovir-diphosphate (TFV-DP), **(C)** emtricitabine (FTC) and tenofovir-diphosphate (FTC-TP).

first dose to steady state, which can be used to reflect adherence levels over the past 1–3 months (*21,22*). Compared to TFV-DP, FTC-TP has a shorter $T_{1/2}$; therefore, its detectable levels could reflect recent dosing (*22*). Owing to this, if the concentrations of both TFV-DP and FTC-TP are high, the patient would have been in good adherence for a certain period of time. In the case of a low TFV-DP with high FTC-TP concentration, it could be an indication that the patient stopped taking PrEP in the past only recently became adherent. In contrast, that is, a patient with a high TFV-DP but low FTC-TP concentration, it could be assumed that such a patient has been adherent for a long time but has recently stopped taking PrEP. For the latter subgroup and those with undetectable levels of both drugs, counseling could be useful to further investigate the underlying reasons. Thus, intracellular TFV-DP and FTC-TP could serve as objective measures to assist clinicians in delivering patient-specific PrEP monitoring interventions.

This study had several limitations. First, the method used to measure intracellular TFV-DP and FTC-TP still needs to be standardized since the concentration of active metabolites, which could be as low as fmol, interferes with the background signal. Second, only FTC/TAF was examined in the plasma samples; however, FTC/TDF should also be analyzed. Third, the samples used in this study were obtained from patients who administered BIC/FTC/TAF. No drug interactions between BIC and FTC/TAF were reported, and in a predefined protocol, the samples were not specific for the FTC/TAF PK study.

In conclusion, intracellular FTV-DP and FTC-TP in DBS could be helpful indicators for monitoring PrEP adherence, due to their reflection prolonged plasma circulation. In addition to the clear advantages of DBS over conventional plasma analysis in terms of sampling and transportation, this study provides additional evidence to the current body of literature for its application in daily practice.

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