Journal of Microbiology, Immunology and Infection 58 (2025) 414–421

Contents lists available at ScienceDirect

Journal of Microbiology, Immunology and Infection

journal homepage: www.e-jmii.com

Measurements of tenofovir-diphosphate and emtricitabine-triphosphate concentrations in dried blood spots of people receiving pre-exposure prophylaxis for HIV with co-formulated tenofovir disoproxil fumarate and emtricitabine

Ya-Ting Lin [a,b,j], Wang-Da Liu [c,d,j], Chih-Ning Cheng [a,b], Wen-Chi Chang [e], Chia-Chi Chuang [e], Hsin-Yun Sun [c], Kuan-Yin Lin [c], Yu-Shan Huang [c], Pei-Ying Wu [f], Ling-Ya Chen [f], Hsi-Yen Chang [f], Yu-Zhen Luo [f], Yi-Ting Chen [f], Wen-Chun Liu [c], Yi-Ching Su [c], Guei-Chi Li [c], Chien-Ching Hung [c,g,h,**], Ching-Hua Kuo [a,b,i,*]

[a] School of Pharmacy, National Taiwan University College of Medicine, Taipei, Taiwan
[b] The Metabolomics Core Laboratory, Center of Genomic and Precision Medicine, National Taiwan University, Taipei, Taiwan
[c] Department of Internal Medicine, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan
[d] Department of Medicine, National Taiwan University Cancer Center, Taipei, Taiwan
[e] The Tenth Core Lab, Department of Medical Research, National Taiwan University, Taipei, Taiwan
[f] Center of Infection Control, National Taiwan University Hospital, Taipei, Taiwan
[g] Department of Tropical Medicine and Parasitology, National Taiwan University College of Medicine, Taipei, Taiwan
[h] Department of Internal Medicine, National Taiwan University Hospital Yunlin Branch, Yunlin, Taiwan
[i] Department of Pharmacy, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan

ARTICLE INFO

Keywords:
Men who have sex with men
Antiretroviral therapy
nucleos(t)ide reverse transcriptase inhibitor
Adherence
Liquid chromatography–mass spectrometry

ABSTRACT

Background/purpose(s): Data regarding the concentrations of tenofovir-diphosphate (TFV-DP) and emtricitabine-triphosphate (FTC-TP) in the Asian population receiving pre-exposure prophylaxis (PrEP) for HIV with tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) (TDF/FTC) are limited, and the associations between the frequency of TDF/FTC administration and drug concentration among people receiving on-demand PrEP remain unclear.

Methods: Fifty-seven participants receiving daily TDF/FTC and 113 participants receiving on-demand TDF/FTC were enrolled in this study. The concentrations of TFV-DP and FTC-TP were measured in dried blood spots using liquid chromatography–mass spectrometry.

Results: Thirty-six (62.2 %) daily PrEP users and 38 (33.6 %) on-demand PrEP users achieved TFV-DP concentrations ≥700 fmol/punch. Higher proportions of undetectable FTC-TP were observed in participants whose TFV-DP concentrations were ≤350 fmol/punch, regardless of the frequency of TDF/FTC administration. In participants who used on-demand PrEP, the TFV-DP and FTC-TP concentrations were moderately correlated with the TDF/FTC tablets taken when sampling was performed within 12–24 h after the last dose of TDF/FTC ($R = 0.63$, $p = 0.006$ and $R = 0.75$, $p = 0.0005$). In addition, on-demand PrEP users who had received 8 tablets within the last 28 days had a median TFV-DP concentration similar to that of those participants who had received 16 tablets (544.6 vs. 556.9 fmol/punch, $p > 0.99$).

Conclusions: These results underscore the importance of well-controlled sampling times for obtaining reliable TFV-DP and FTC-TP concentrations to estimate the adherence and effectiveness of on-demand PrEP.

* Corresponding author. School of Pharmacy, National Taiwan University College of Medicine, Rm. 418, 4th F, No. 33, Linsen S. Rd., Zhongzheng Dist., Taipei, Taiwan.
** Corresponding author. Department of Internal Medicine, National Taiwan University Hospital, 7 Chung-Shan South Road, Taipei, Taiwan.
E-mail addresses: hcc0401@ntu.edu.tw (C.-C. Hung), kuoch@ntu.edu.tw (C.-H. Kuo).
[j] Lin YT and Liu WD contributed equally to the work.

https://doi.org/10.1016/j.jmii.2025.03.002
Received 8 August 2024; Received in revised form 22 February 2025; Accepted 1 March 2025
Available online 8 March 2025

1. Introduction

Pre-exposure prophylaxis (PrEP) with daily co-formulated tenofovir disoproxil fumarate and emtricitabine (TDF/FTC) has been approved by the U.S. Food and Drug Administration (FDA) to prevent HIV infection among men who have sex with men (MSM) and heterosexual men and women.[1] The iPrEx study first reported that daily use of PrEP with TDF/FTC resulted in a 44 % reduction in HIV acquisition among MSM and transgender women.[2] The PROUD study reported that daily PrEP conferred even greater protection, with an 86 % reduction in HIV acquisition.[3] Moreover, the IPERGAY study, which challenged the concept of daily PrEP, reported similar efficacies between on-demand and daily PrEP.[4] On-demand PrEP has a lower pill burden, which is advantageous for people who find it difficult to use daily PrEP.[5,6] However, while PrEP is highly effective for HIV prevention, poor adherence may diminish its effectiveness due to a suboptimal drug concentration.[7]

The use of traditional measurements, such as self-reporting, pill counts, and HIV viral loads, remains a challenge in adherence monitoring among people with HIV who receive antiretroviral therapy.[8] The determination of drug concentrations may represent an alternative to adherence measurements. This approach provides an advantage in predicting efficacy or effectiveness compared to traditional adherence measurement methods. To date, several studies have attempted to evaluate the association between the effectiveness of PrEP and the concentrations of tenofovir-diphosphate (TFV-DP) and emtricitabine-triphosphate (FTC-TP),[2,9-11] which are active metabolites formed by the respective prodrugs of TDF and FTC.[12] Previous studies have suggested that a TFV-DP concentration ≥700 fmol/punch in dried blood spots (DBSs) will be associated with a 100 % reduction in preventing HIV acquisition, while concentrations of 350–700 fmol/punch provide approximately 90 % protection against HIV acquisition. As few as 4-7 tablets per week can achieve a TFV-DP concentration ≥700 fmol/punch.[11] In addition, the half-lives of TFV-DP and FTC-TP in DBSs are 17.1 and 1.5 days, respectively, and their respective concentrations may be surrogate markers of long-term and short-term adherence.[13-16] Although clinical data support the use of TFV-DP and FTC-TP concentrations in DBSs to assess the effectiveness of PrEP for HIV, such data are limited in the Asian population.[17]

While several clinical studies have reported similar efficacies of on-demand and daily PrEP for the prevention of HIV infection,[4,6,18,19] it is unknown whether the concentrations of TFV-DP and FTC-TP reach the recommended levels for conferring adequate protection in people receiving on-demand PrEP with TDF/FTC. A previous study estimated the number of tablet taken using TFV-DP concentrations among participants receiving on-demand PrEP.[20] However, the actual number of tablets taken may differ from the estimated number. More research is necessary to determine the concentrations of TFV-DP and FTC-TP among people taking on-demand PrEP and to investigate whether target concentrations known to confer 100 % protection against HIV can be achieved using on-demand PrEP. Therefore, this study analyzed the TFV-DP and FTC-TP concentrations in DBSs from individuals receiving daily or on-demand PrEP with TDF/FTC. This study provides data regarding the distribution of these concentrations in the Taiwanese population and the associations between concentrations and the number of TDF/FTC tablets taken by individuals receiving on-demand PrEP with different dosing frequencies and sampling times.

2. Methods

2.1. Study design and participants

Men who have sex with men receiving either daily or on-demand PrEP between October 23, 2018 and August 31, 2023 were enrolled in this study. Participants with chronic hepatitis B were excluded from the study. All participants in the daily PrEP group had been taking TDF/FTC on a daily basis for at least one month before enrollment. Participants in the on-demand PrEP group took two tablets of TDF/FTC within 2–24 h before sexual intercourse, followed by one tablet each at 24 and 48 h after the first dose.[4,21]

2.2. Procedure

Each participant completed a self-administered questionnaire, in which data regarding risky sexual behavior, the type of PrEP use (daily vs. on-demand), and the length of time between the last dose of TDF/FTC and the study visit were obtained. Blood was drawn for both DBS collection and HIV testing.

A 15-μL aliquot of a whole blood sample originally collected using EDTA tubes was used for the DBS samples. The DBS was collected on a Whatman 903 card. The DBS cards were dried at room temperature in the dark for at least 4 h or overnight and were stored in double-layer ziplock bags with a desiccant. The cards were then stored at −20 °C until analysis.

2.3. Quantification of TFV-DP and FTC-TP concentrations in DBSs

This study was conducted by adopting an on-spot internal standard addition strategy followed by a modified indirect method according to previous studies for more accurate measurements of TFV-DP and FTC-TP concentrations in DBS samples.[22-24] Briefly, 5 μL of internal standard (200 ng mL^{-1} tenofovir-d6 diphosphate) was spotted onto each DBS sample and dried at room temperature in the dark for 30 min. The whole spot (8-mm diameter) was collected using several punches. The punched spots were extracted by adding 1000 μL of 70 % methanol using a Geno/Grinder 2010 (SPEX Sample Prep, Metuchen, NJ, USA) for 15 min at 1000 rpm. The supernatant underwent solid-phase extraction (SPE) using cartridges (Waters AccellTM Plus QMA Cartridge, 3 cc, 500 mg) to isolate TFV-DP and FTC-TP. KCl solutions were used for washing (5 mL of 75 mM solution and 6 mL of 90 mM solution) and elution of the target analytes (2 mL of 1M solution). Then, 150 μL of acid phosphatase (10 units, pH 5), which was diluted with 1 M sodium acetate to a total volume of 20 mL, was added to the eluted solution and incubated at 37 °C for 60 min. After incubation, 20 μL of the internal standard (20 ng mL^{-1} FTC-$^{13}C^{15}N_2$) and 100 μL of 12 % trifluoroacetic acid were added. The sample was added to another cartridge (Waters OASIS® HLB Extraction Cartridge, 3 cc, 60 mg). A 1% trifluoroacetic acid solution (2 mL) was added to wash the sample, and 100 % methanol (1 mL) was used to elute the target analytes. The eluted sample was dried using a gentle stream of nitrogen. The residue was reconstituted with 100 μL of deionized water and analyzed using a UHPLC-MS/MS system. The measured concentrations were normalized to 3 mm punches by calculating the area of the blood spot, represented as fmol/punch. The lower limit of quantification (LLOQ) was 85 fmol/punch for TFV-DP and 100 fmol/punch for FTC-TP. Chemicals, detailed UHPLC-MS/MS analytical parameters and method validation results are provided in the supplementary material (Section S-1, Section S-2, Table S1, and Table S2).

2.4. Statistical analysis

The chi-squared test and logistic regression analyses were used to compare undetectable FTC-TP concentrations based on different TFV-DP concentration categories. Pearson's correlation analysis was used to identify associations between the concentrations and the number of TDF/FTC tablets taken each week. Differences in the concentrations of TDF/FTC based on the number of tablets taken per month were tested using an analysis of variance (ANOVA). Post hoc pairwise comparisons were also conducted using Bonferroni's adjustment. All statistical analyses were performed using SAS (version 9.4, SAS Institute, Cary, NC, USA). Boxplots and line graphs were constructed using GraphPad Prism (version 8.0, GraphPad Software, La Jolla, CA, USA).

3. Results

3.1. Study participants and baseline characteristics

A total of 170 male participants were enrolled in this study, including 57 in the daily PrEP group and 113 in the on-demand PrEP group. TFV-DP and FTC-TP concentrations in DBSs were measured for all participants. Among the participants in the on-demand PrEP group, 45 (78.9 %) recorded the time of the last PrEP regimen taken before blood sampling and 24 (42.1 %) recorded the number of tablets taken per month (Fig. 1).

Table 1 presents the baseline characteristics of the participants who used daily and those who used on-demand PrEP. The median age was 29.0 years and the median weight was 70.0 kg for the whole cohort; and the median body mass index of the participants was 23.4 kg/m^2 in the daily PrEP group and 23.3 kg/m^2 in the on-demand PrEP group. Although the sexual orientation, the number of regular sexual partners, and the presence of sexual partners with HIV infection were different between daily and on-demand PrEP groups, none of the participants enrolled in this study seroconverted for HIV during the study observation period.

3.2. TFV-DP and FTC-TP concentrations in DBSs among participants

In the daily PrEP group, the median TFV-DP concentration was 803.9 fmol/punch (interquartile range [IQR], 473.1–1015.0 fmol/punch) and the median FTC-TP concentration was 261.0 fmol/punch (IQR, 184.6–383.1 fmol/punch). In the on-demand PrEP group, the median TFV-DP concentration was 488.6 fmol/punch (IQR, 305.1–811.4 fmol/punch) and the median FTC-TP concentration was 297.6 fmol/punch (IQR, 206.3–387.5 fmol/punch) (Fig. 2A).

The distributions of TFV-DP concentration in participants in the daily and on-demand PrEP groups are shown in Fig. 2B by following the four categories defined in a previous study,[11] which included less than 350 fmol/punch, 350-700 fmol/punch, 700-1250 fmol/punch, and greater than 1250 fmol/punch. FTC-TP concentrations were classified as undetectable or quantifiable according to the LLOQ level at 100 fmol/punch based on the results of a previous study.[15]

Among the 57 daily PrEP participants, 36 (63.2 %) had TFV-DP concentrations >700 fmol/punch and 9 (15.8 %) had TFV-DP concentrations <350 fmol/punch. Among the 113 on-demand PrEP participants, 38 (33.6 %) had TFV-DP concentrations >700 fmol/punch and 34 (30.1 %) had concentrations <350 fmol/punch (Fig. 2B). Table 2 shows the proportions of undetectable and quantifiable FTC-TP concentrations across the four categories of TFV-DP concentration in DBSs. Undetectable FTC-TP concentrations were associated with a TFV-DP concentration <350 fmol/punch in the daily ($p < 0.0001$) and on-demand ($p = 0.03$) PrEP groups. Undetectable FTC-TP concentrations were more likely to be observed in the participants with TFV-DP concentrations <350 fmol/punch in the daily PrEP group (odds ratio, 48.00; 95 % confidence interval [95 % CI], 6.62–347.47; $p = 0.0001$) and on-demand PrEP group (odds ratio, 6.56; 95 % CI, 1.58–27.22; $p = 0.0009$).

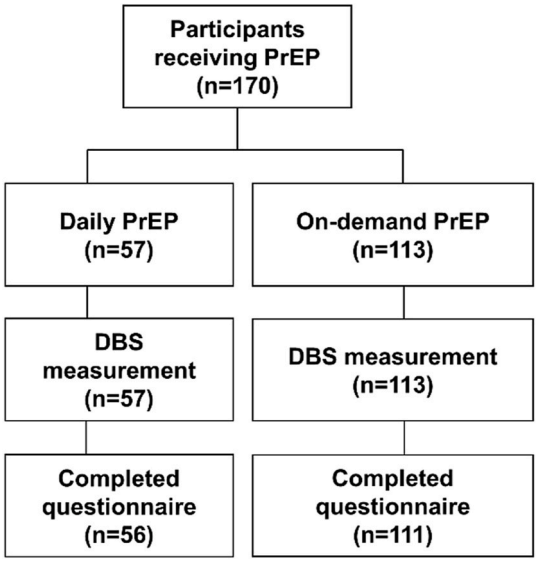

Fig. 1. Flowchart of the participants using daily and on-demand PrEP.

Table 1
Baseline characteristics of study participants using daily and on-demand PrEP.

Characteristics	Daily (n = 57)	On-demand (n = 113)	P value
Age (years)	29.0 (26.0–31.0)	29.0 (26.0–32.0)	0.23
Weight (kg)	70.0 (63.0–75.0)	70.0 (64.0–78.0)	0.55
BMI (kg/m^2)	23.4 (21.2–24.8)	23.3 (21.6–25.8)	0.69
Sexual orientation			0.02
Available dataset (n)	56	111	
Men who have sex with men	46 (82)	105 (95)	
Heterosexuals	10 (18)	6 (5)	
Number of regular sexual partners in the past 3 months			0.04
Available dataset (n)	56	111	
≤5	47 (84)	102 (92)	
6-10	3 (5)	7 (6)	
≥11	6 (11)	2 (2)	
Anal sex without condom use			0.64
Available dataset (n)	56	111	
Yes	40 (71)	83 (75)	
No	16 (29)	28 (25)	
Fixed partner			0.10
Available dataset (n)	56	110	
Yes	23 (41)	60 (55)	
No	33 (59)	50 (45)	
Sexual partner with HIV infection			0.04
Available dataset (n)	56	111	
Yes	11 (20)	22 (20)	
No	37 (66)	85 (77)	
Unknown	8 (14)	4 (3)	
Former or current use of recreational drugs			0.35
Available dataset (n)	56	111	
Yes	2 (4)	8 (7)	
No	54 (96)	103 (93)	

Data are presented as median (IQR) or number (percentage).

3.3. Correlations between TDF/FTC tablets and TFV-DP and FTC-TP concentrations

Data from 45 participants in the on-demand PrEP group were used to investigate the associations between the number of TDF/FTC tablets taken each week and the TFV-DP and FTC-TP concentrations. These participants were categorized based on the number of TDF/FTC tablets taken per week: 1-3 tablets (n = 5), 4-6 tablets (n = 22), and 7 tablets (n = 18). The median TFV-DP concentrations for the 1-3, 4-6, and 7 tablet groups were 119.3 fmol/punch, 408.1 fmol/punch, and 774.4 fmol/punch, respectively (Fig. 3A). The results showed that TFV-DP concentration increased when more TDF/FTC tablets were taken weekly.

In contrast, FTC-TP concentrations did not increase when more TDF/FTC tablets were taken, particularly in the group of participants taking 4-6 tablets per week. This could be attributed to the fact that some samples were collected longer after the last TDF/FTC tablet taken. After excluding 9 samples in the 4-6 tablet group that were collected >60 h after TDF/FTC administration, the FTC-TP concentrations also increased with increasing TDF/FTC tablets taken (Fig. 3B). The median FTC-TP concentrations for the 1-3, 4-6, and 7 tablet groups were 207.5 fmol/punch, 255.0 fmol/punch, and 376.6 fmol/punch, respectively. The

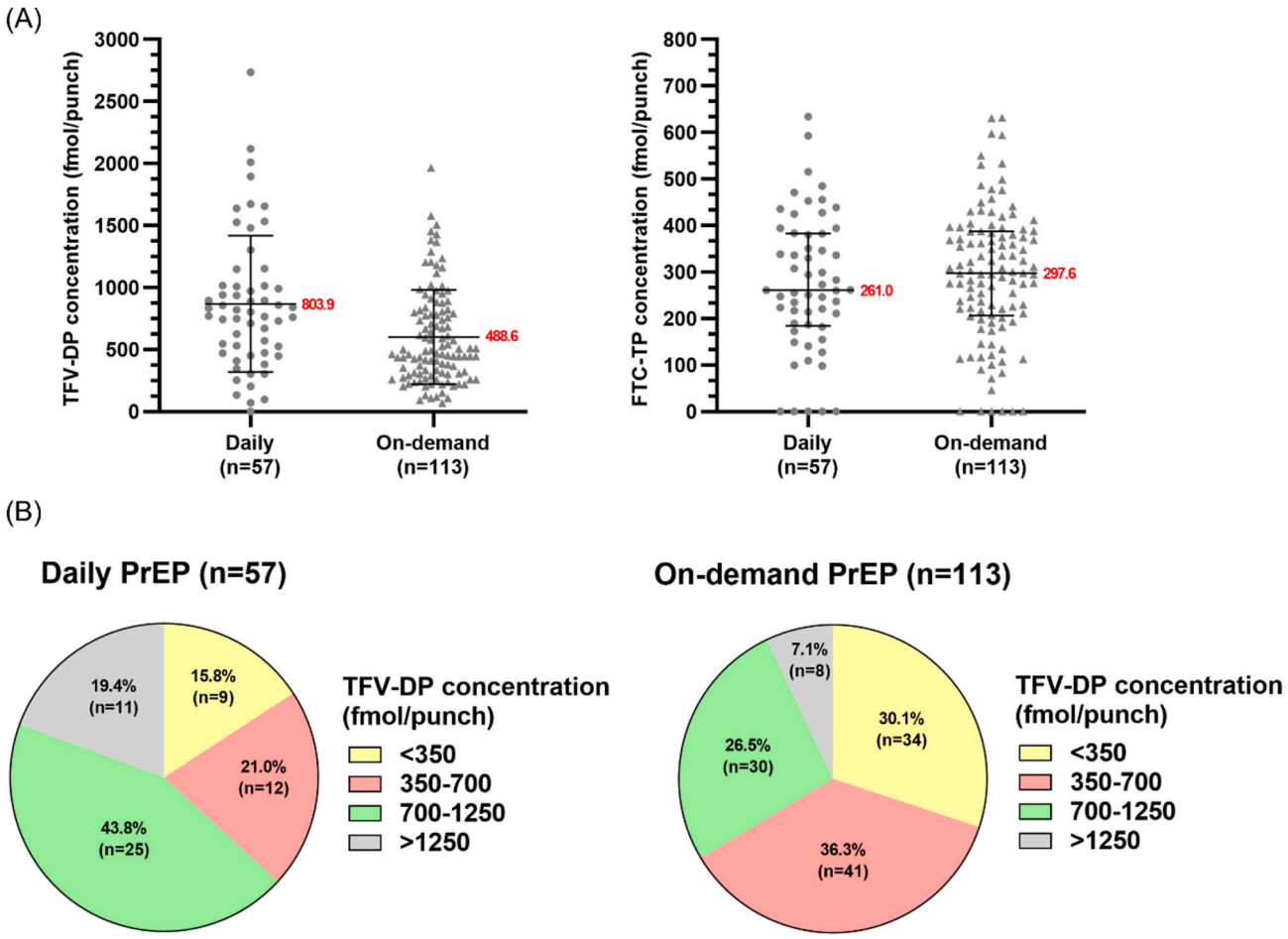

Fig. 2. (A) The TFV-DP and FTC-TP concentration distributions among participants using daily and on-demand PrEP are shown. The median concentrations are shown on the right side of the median line. (B) The proportions of four categories of TFV-DP concentrations among participants using daily and on-demand PrEP are shown. The percentages for each group are displayed in the pie chart.

Table 2
Undetectable and quantifiable FTC-TP concentrations in DBSs.

		TFV-DP (fmol/punch)				
Daily PrEP (n = 57)	FTC-TP (fmol/punch)	<350, n (%) (n = 9)	350-700, n (%) (n = 12)	700-1250, n (%) (n = 25)	>1250, n (%) (n = 11)	P
	Undetectable (<100)	6 (67)	0 (0)	1 (4)	0 (0)	
	Quantifiable (>100)	3 (33)	12 (100)	24 (96)	11 (100)	<0.0001
On-demand PrEP (n = 113)	FTC-TP (fmol/punch)	<350, n (%) (n = 34)	350-700, n (%) (n = 41)	700-1250, n (%) (n = 30)	>1250, n (%) (n = 8)	P
	Undetectable (<100)	7 (21)	1 (2)	2 (7)	0 (0)	
	Quantifiable (>100)	27 (79)	40 (98)	28 (93)	8 (100)	0.0313

TFV-DP concentration trend did not differ when the nine samples were excluded in a subsequent analysis.

Considering that the timing of blood sample collection after the last TDF/FTC tablet was taken might affect FTC-TP concentrations, we further categorized the 45 included participants into five groups based on the interval between the last dose taken and sample collection: 0-12 h, 12-24 h, 24-36 h, 36-72 h, and >72 h. We then studied the relationship between TFV-DP and FTC-TP concentrations and TDF/FTC tablets taken per week in each category. The TFV-DP ($R = 0.63$; $p = 0.006$) and FTC-TP ($R = 0.75$; $p = 0.0005$) concentrations increased as the dosage increased in the 12-24 h group, exhibiting a moderate correlation (Fig. 4). Therefore, blood samples were collected within 12–24 h after the last dosing to assess the impact of the number of TDF/FTC tablets taken per month on TFV-DP and FTC-TP concentrations in the on-demand PrEP group.

3.4. TFV-DP and FTC-TP concentrations among participants using on-demand PrEP with different tablets per month

We collected DBSs from 24 participants taking 4, 8, and 16 tablets per month within 12–24 h post-dose. The TFV-DP and FTC-TP concentrations increased with the number of TDF/FTC tablets taken (Fig. 5). The median TFV-DP concentration in the 4, 8, and 16 tablets/month groups were 220.9 fmol/punch, 544.6 fmol/punch, and 556.9 fmol/punch, respectively. Seventeen participants had TFV-DP concentrations >350 fmol/punch, including 1 (16.6 %) in the 4 tablets/month group, 7 (87.5 %) in the 8 tablets/month group, and 9 (90.0 %) in the 16 tablets/month group. The FTC-TP concentration was quantifiable in all participants. The median FTC-TP concentration in the 4, 8, and 16 tablets/month groups was 224.7 fmol/punch, 321.1 fmol/punch, and 364.2 fmol/punch, respectively. There were no significant differences in TFV-

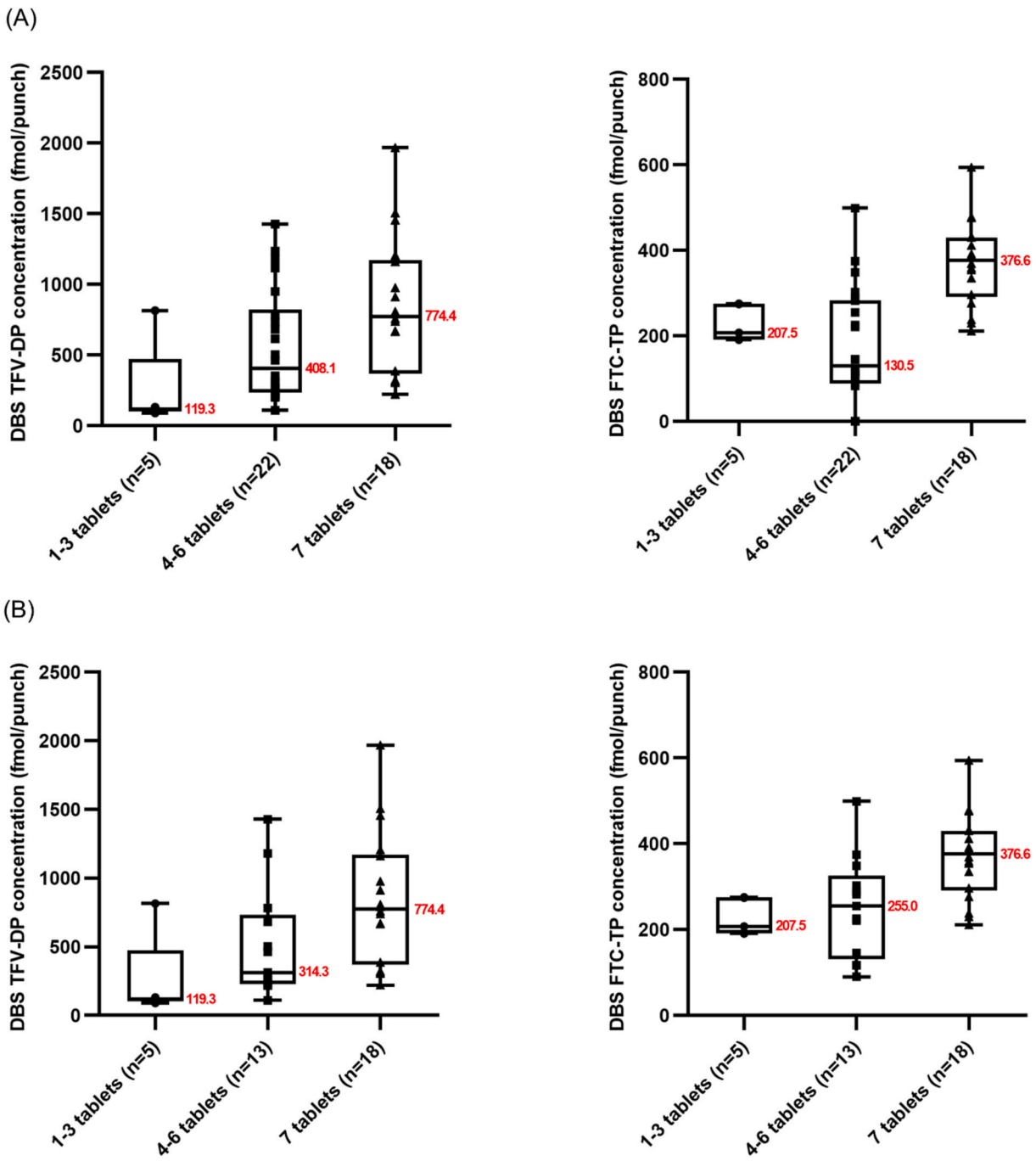

Fig. 3. The concentrations of TFV-DP and FTC-TP in DBSs obtained from participants using on-demand PrEP were categorized into three groups according to their weekly tablet intake. Boxplots including data before (A) and after (B) excluding samples collected >60 h after the last dose of PrEP are shown. The median value for each group is presented on the right side of the boxplot.

DP or FTC-TP concentrations between the participants taking 8 tablets/month and those taking 16 tablets/month (Fig. 5).

4. Discussion

This study investigated the TFV-DP and FTC-TP concentrations in DBSs from Taiwanese people without HIV who received TDF/FTC as daily or on-demand PrEP for HIV. The FTC-TP concentrations were affected by sampling time among on-demand PrEP users. These results suggest that sample collection within 12–24 h after dosing could obtain results to more reliably assess the adherence among on-demand PrEP users. In addition, the TFV-DP and FTC-TP concentrations were similar between the participants taking 8 tablets/month and those taking 16 tablets/month in the on-demand PrEP group.

In this study, we found that 9 of 57 (15.8 %) daily PrEP participants had TFV-DP concentrations below 350 fmol/punch, while 36 out of 57 (63.2 %) had concentrations greater than 700 fmol/punch. A real-world PrEP clinical study showed that 73.9 % of daily PrEP participants had a TFV-DP concentration greater than 700 fmol/punch and 11.5 % had a concentration below 350 fmol/punch.[25] Our study shows a slight difference in the proportions of participants with low vs. high TFV-DP concentrations compared to those observed in the previous study. In addition to racial or ethnic differences in pharmacokinetics, the TFV-DP concentrations are found to be higher in older participants, those with a

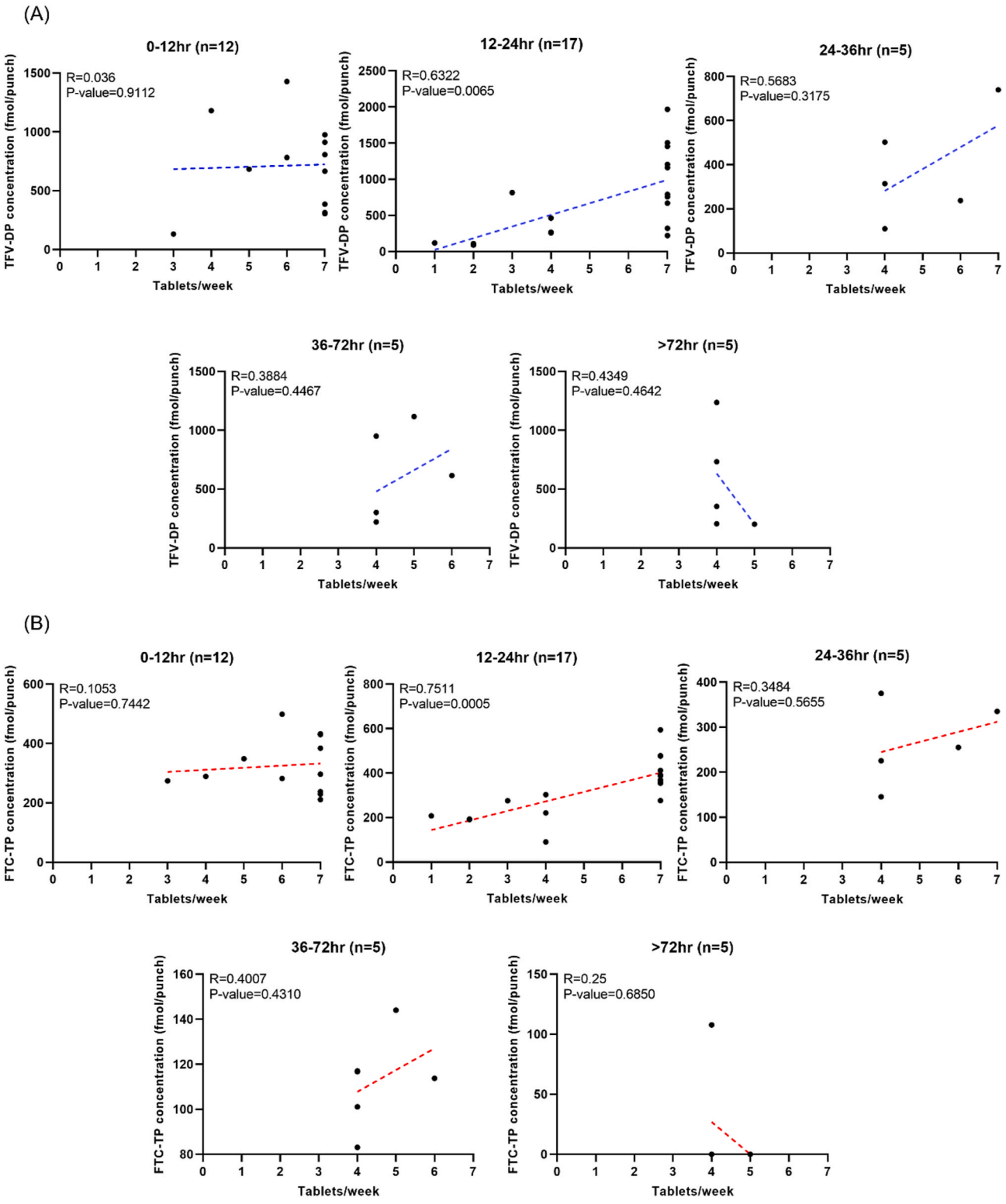

Fig. 4. The correlations between weekly tablets and the (A) TFV-DP and (B) FTC-TP concentrations are shown.

lower body mass index, those engaged in sex without condom use, and those with sexual partners with HIV.[11,25-27] The difference in TFV-DP distribution between our study and others could be due to the proportions of participants aged over 35 years (27.7 %). Additionally, previous studies had higher proportions of participants with HIV-positive sexual partners (39.4 %) compared to our study (11[20.0 %] for daily PrEP group). A recent study conducted in Asia reported that 169 out of 191 (88.4 %) of DBS samples from daily PrEP users had TFV-DP concentrations >700 fmol/punch,[17] which was higher than that in the current study. The previous study primarily focused on developing the analytical method and the characteristics of the PrEP participants were not reported, making it difficult to identify potential factors contributing to the concentration differences observed.[17]

The median TFV-DP concentration of the participants in the on-

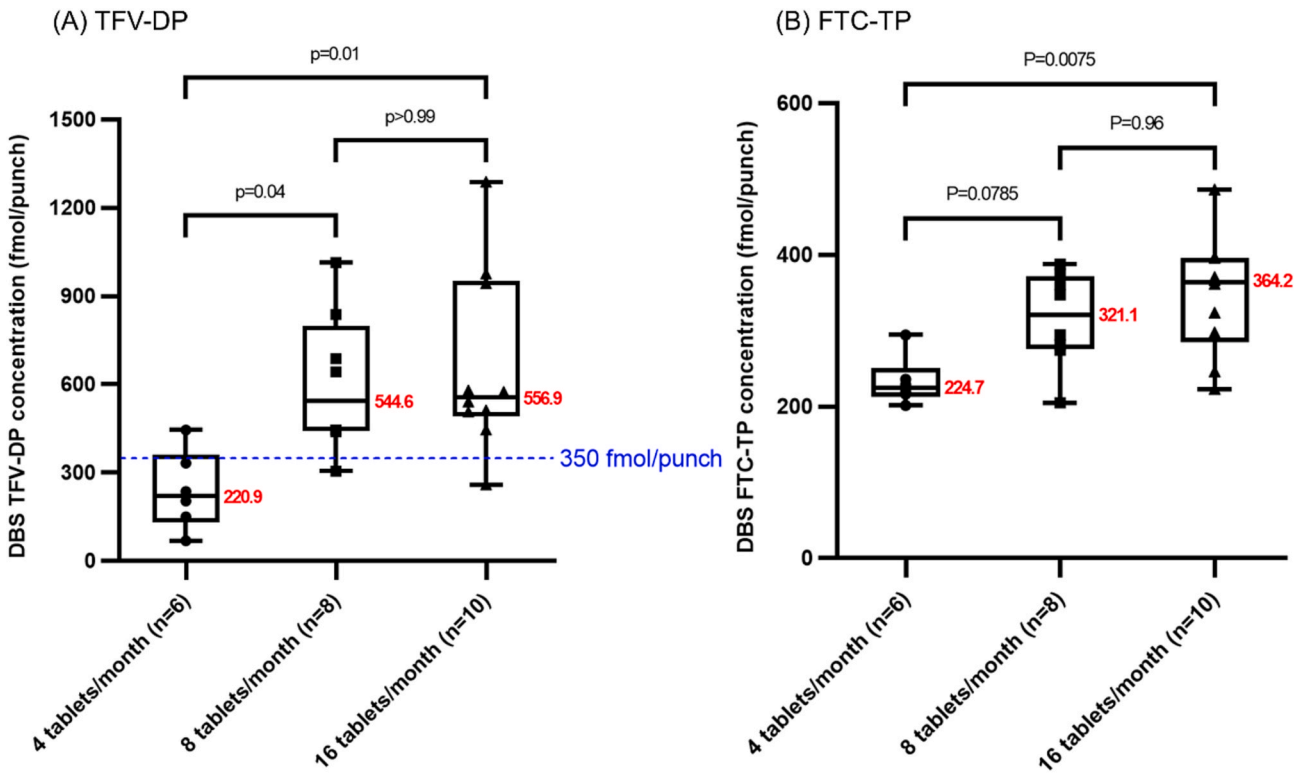

Fig. 5. TFV-DP and FTC-TP concentrations in DBSs of on-demand PrEP users taking 4, 8, or 16 TDF/FTC tablets per month are shown. The median value for each group is presented on the right side of the boxplot.

demand PrEP group in the current study was 488.6 (IQR, 305.1–811.4) fmol/punch, which was similar to that reported in the ANRS IPERGAY clinical trial and AMPrEP project in Amsterdam, which showed that the median TFV-DP concentration was 517 (IQR, 128-868) fmol/punch and 591 (IQR, 270-896) fmol/sample, respectively.[19,20] The proportion of on-demand PrEP users with a TFV-DP concentration >700 fmol/punch in the current study (33.6 %) was similar to that reported in the ANRS IPERGAY clinical trial (39 %), which reported a 97 % reduction in HIV incidence among participants using on-demand PrEP.[20,21]

We further evaluated the association between TFV-DP and FTC-TP concentrations and the number of TDF/FTC tablets taken per week among the on-demand PrEP participants. We observed that a longer interval between the last TDF/FTC tablet taken and blood sample collection affected the FTC-TP concentration. Castillo-Mancilla et al. determined that the half-life of FTC-TP in DBSs was approximately 1.5 days and suggested that samples collected within 48 h after taking TDF/FTC exhibited quantifiable FTC-TP levels and samples collected >96 h after dosing had unquantifiable FTC-TP levels.[15] Though concentrations may be affected by sampling time, the association between concentrations and the number of TDF/FTC tablets taken has not been adequately investigated in previous studies. In the current study, the concentrations of TFV-DP and FTC-TP moderately correlated with the timing of samples collected within 12–24 h, 24-36 h and 36-72 h after dosing. However, due to the sample sizes in 24–36-h and 36-72-h groups were relatively small; further studies of larger sample sizes are required to investigate this relationship. Nevertheless, the findings of our study suggest controlling the sample collection time within 12–24 h after the last dose can obtain more reliable concentration measurements.

The TFV-DP concentration of participants taking 16 TDF/FTC tablets/month was similar to that of those taking 8 tablets/month in the current study. In a previous study, on-demand PrEP users taking a median of 15 TDF/FTC tablets/month reported an 86 % relative reduction in HIV acquisition.[4] However, the previous study did not report data regarding the TFV-DP and FTC-TP concentrations. To bridge our observation with their study, we adapted a TFV-DP concentration cutoff of 350 fmol/punch with approximately 90 % protection in the current study, which closely aligns with a previous report.[4,11] In our study, 9/10 (90.0 %) participants taking 16 tablets per month had TFV-DP concentrations higher than 350 fmol/punch. The proportions were similar between the participants taking 8 tablets per month (7/8, 87.5 %) and those taking 16 tablets per month. While our study showed similar proportions of participants achieving TFV-DP concentrations higher than 350 fmol/punch with taking 16 tablets vs. 8 tablets, additional clinical data are needed to substantiate the effectiveness of taking 8 TDF/FTC tablets/month for HIV prevention.

This study has several limitations. First, the number of participants who used daily or on-demand PrEP regimens remained relatively small. Second, the frequency of PrEP use and the time of the last dose relied on the participants' recall, potentially contributing to study bias. Third, the participants' adherence to daily or on-demand PrEP was not verified. Finally, all participants were recruited from a single medical hospital, which limits the generalizability of the findings.

In conclusion, the results of this study suggest that DBS collection within 12–24 h after the last dose of TDF/FTC yields more reliable concentrations of TFV-DP and FTC-TP to predict adherence among on-demand PrEP users. Furthermore, this study revealed that the distribution of the TFV-DP concentration of on-demand PrEP participants taking 8 tablets/month was similar to that of on-demand PrEP participants taking 16 tablets/month.

CRediT authorship contribution statement

Ya-Ting Lin: Writing – original draft, Methodology, Investigation. **Wang-Da Liu:** Writing – review & editing, Methodology, Conceptualization. **Chih-Ning Cheng:** Investigation. **Wen-Chi Chang:** Investigation. **Chia-Chi Chuang:** Investigation. **Hsin-Yun Sun:** Conceptualization. **Kuan-Yin Lin:** Conceptualization. **Yu-Shan Huang:** Conceptualization. **Pei-Ying Wu:** Investigation. **Ling-Ya Chen:**

Investigation. **Hsi-Yen Chang:** Investigation. **Yu-Zhen Luo:** Investigation. **Yi-Ting Chen:** Investigation. **Wen-Chun Liu:** Investigation. **Yi-Ching Su:** Investigation. **Guei-Chi Li:** Investigation. **Chien-Ching Hung:** Writing – review & editing, Conceptualization. **Ching-Hua Kuo:** Writing – review & editing, Conceptualization.

Ethics approval and consent to participate

The study was approved by the National Taiwan University Hospital Research Ethics Committee (NTUH REC No. 201712175RINA and 202302069RINB). All participants provided written informed consent.

Funding

This study was supported by the Taiwan Centers for Disease Control.

Declaration of competing interest

The authors have no conflicts of interest to declare.

Acknowledgments

The authors thank people who participated in this study at the National Taiwan University Hospital. The authors acknowledge National Science and Technology Council of Taiwan (NSTC 110-2113-M-002-006-MY3), Ministry of Education, National Taiwan University (NTU) School of Pharmacy Endowment Fund in support of the Platform for Clinical Mass Spectrometry and NMR Structure Elucidation. Y.T.L. was supported by a scholarship from the XinChen Medical Research Foundation, Taiwan.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2025.03.002.

References

1. Okwundu CI, Uthman OA, Okoromah CA. Antiretroviral pre-exposure prophylaxis (PrEP) for preventing HIV in high-risk individuals. *Cochrane Database Syst Rev*. 2012; 2012(7), CD007189.
2. 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;363:2587–2599.
3. McCormack S, Dunn DT, Desai M, et al. Pre-exposure prophylaxis to prevent the acquisition of HIV-1 infection (PROUD): effectiveness results from the pilot phase of a pragmatic open-label randomised trial. *Lancet*. 2016;387:53–60.
4. Molina JM, Capitant C, Spire B, et al. On-demand preexposure prophylaxis in men at high risk for HIV-1 infection. *N Engl J Med*. 2015;373:2237–2246.
5. Beymer MR, Holloway IW, Pulsipher C, Landovitz RJ. Current and future PrEP medications and modalities: on-demand, injectables, and topicals. *Curr HIV AIDS Rep*. 2019;16:349–358.
6. Stansfield SE, Moore M, Boily MC, Hughes JP, Donnell DJ, Dimitrov DT. Estimating benefits of using on-demand oral prep by MSM: a comparative modeling study of the US and Thailand. *EClinicalMedicine*. 2023;56, 101776.
7. Riddell Jt, Amico KR, Mayer KH. HIV preexposure prophylaxis: a review. *JAMA*. 2018;319:1261–1268.
8. Castillo-Mancilla JR, Haberer JE. Adherence measurements in HIV: new advancements in pharmacologic methods and real-time monitoring. *Curr HIV AIDS Rep*. 2018;15:49–59.
9. Adams JL, Sykes C, Menezes P, et al. Tenofovir diphosphate and emtricitabine triphosphate concentrations in blood cells compared with isolated peripheral blood mononuclear cells: a new measure of antiretroviral adherence? *J Acquir Immune Defic Syndr*. 2013;62:260–266.
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, 151ra25.
11. 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;14:820–829.
12. Ray AS, Fordyce MW, Hitchcock MJ. Tenofovir alafenamide: a novel prodrug of tenofovir for the treatment of human immunodeficiency virus. *Antivir Res*. 2016; 125:63–70.
13. Castillo-Mancilla JR, Zheng JH, Rower JE, et al. Tenofovir, emtricitabine, and tenofovir diphosphate in dried blood spots for determining recent and cumulative drug exposure. *AIDS Res Hum Retrovir*. 2013;29:384–390.
14. Castillo-Mancilla JR, Searls K, Caraway P, et al. Tenofovir diphosphate in dried blood spots as an objective measure of adherence in HIV-infected women. *AIDS Res Hum Retrovir*. 2015;31:428–432.
15. Castillo-Mancilla J, Seifert S, Campbell K, et al. Emtricitabine-triphosphate in dried blood spots as a marker of recent dosing. *Antimicrob Agents Chemother*. 2016;60: 6692–6697.
16. Frasca K, Morrow M, Coyle RP, et al. Emtricitabine triphosphate in dried blood spots is a predictor of viral suppression in HIV infection and reflects short-term adherence to antiretroviral therapy. *J Antimicrob Chemother*. 2019;74:1395–1401.
17. Tsuchiya K, Hayashi Y, Ryu S, et al. Determination of intracellular tenofovir-diphosphate and emtricitabine-triphosphate concentrations in dried blood spots for pre-exposure prophylaxis adherence. *J Infect Chemother*. 2024;30:876–880.
18. Molina JM, Ghosn J, Assoumou L, et al. Daily and on-demand HIV pre-exposure prophylaxis with emtricitabine and tenofovir disoproxil (ANRS PREVENIR): a prospective observational cohort study. *Lancet HIV*. 2022;9:e554–e562.
19. Jongen VW, Hoornenborg E, van den Elshout MA, et al. Adherence to event-driven HIV PrEP among men who have sex with men in Amsterdam, The Netherlands: analysis based on online diary data, 3-monthly questionnaires and intracellular TFV-DP. *J Int AIDS Soc*. 2021;24, e25708.
20. Goldwirt L, Bauer R, Liegeon G, et al. Estimated pill intake with on-demand PrEP with oral TDF/FTC using TFV-DP concentration in dried blood spots in the ANRS IPERGAY trial. *J Antimicrob Chemother*. 2021;76:2675–2680.
21. Molina JM, Charreau I, Spire B, et al. Efficacy, safety, and effect on sexual behaviour of on-demand pre-exposure prophylaxis for HIV in men who have sex with men: an observational cohort study. *Lancet HIV*. 2017;4:e402–e410.
22. King T, Bushman L, Kiser J, et al. Liquid chromatography-tandem mass spectrometric determination of tenofovir-diphosphate in human peripheral blood mononuclear cells. *J Chromatogr, B: Anal Technol Biomed Life Sci*. 2006;843:147–156.
23. 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.
24. Tsai IL, Kuo CH, Sun HY, et al. An on-spot internal standard addition approach for accurately determining colistin A and colistin B in dried blood spots using ultra high-performance liquid chromatography-tandem mass spectrometry. *J Pharm Biomed Anal*. 2017;145:783–793.
25. Grinsztejn B, Hoagland B, Moreira RI, et al. Retention, engagement, and adherence to pre-exposure prophylaxis for men who have sex with men and transgender women in PrEP Brasil: 48 week results of a demonstration study. *Lancet HIV*. 2018;5: e136–e145.
26. Coyle RP, Morrow M, Coleman SS, et al. Factors associated with tenofovir diphosphate concentrations in dried blood spots in persons living with HIV. *J Antimicrob Chemother*. 2020;75:1591–1598.
27. Castillo-Mancilla JR, Morrow M, Coyle RP, et al. Tenofovir diphosphate in dried blood spots is strongly associated with viral suppression in individuals with human immunodeficiency virus Infections. *Clin Infect Dis*. 2019;68:1335–1342.