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BRIEF REPORT

Lower Urine Tenofovir Concentrations Among Individuals Taking Tenofovir Alafenamide Versus Tenofovir Disoproxil Fumarate: Implications for Point-of-Care Testing

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From directly observed therapy studies, urine tenofovir (TFV) levels were 74% lower when taking tenofovir alafenamide (TAF) vs tenofovir disoproxil fumarate. Urine TFV remains quantifiable across a range of TAF adherence patterns, but a separate point-of-care lateral flow immunoassay with a lower TFV threshold will be needed to support TAF adherence monitoring.

Keywords. directly observed therapy; pharmacokinetics; point-of-care adherence monitoring; tenofovir alafenamide; tenofovir disoproxil fumarate.

Preexposure prophylaxis (PrEP) for those at risk of human immunodeficiency virus (HIV) exposure and treatment-as-prevention for those living with HIV are key strategies for the US Ending the HIV Epidemic initiative [1]. Because PrEP and treatment both rely on strict medication adherence to achieve optimal efficacy, adequate tools to monitor and support antiretroviral adherence are needed to maximize their impact.

Studies have shown that objective pharmacologic metrics of adherence—such as measuring drug levels in dried blood spots (DBSs), plasma, hair, or urine—more accurately predict antiretroviral (ARV) efficacy than self-reported adherence [2]. While traditional pharmacologic measures require time-intensive and expensive mass spectrometry–based testing, antibody-based technologies applied to immunoassays allow for low-cost drug level testing in real time. Adequate adherence to ARVs could be supported by point-of-care (POC) immunoassay monitoring to provide an objective adherence metric for use in routine care settings.

Tenofovir (TFV) is the metabolite of both tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide (TAF), each used frequently for treatment. Both TDF/emtricitabine (FTC) and TAF/FTC (exclusive of vaginal receptive sex) are also approved for PrEP. A novel method to measure urine levels of TFV in patients on TDF using a low-cost, easy-to-perform, antibody-based immunoassay has now been developed [3–5]. When tested against the gold standard of liquid chromatography/tandem mass spectrometry (LC-MS/MS), this assay has proven sensitive/specific (97%–99%) [2] with a high degree of correlation between the 2 techniques (0.92, $P < .001$) [4]. This enzyme-linked immunoassay has been converted to a lateral flow assay [3], like a urine pregnancy test, allowing POC testing of recent TDF ingestion (providing a qualitative yes/no answer as to whether TDF has been taken within the last 5 days).

TAF is primarily metabolized intracellularly, resulting in plasma TFV levels that are approximately 90% lower with TAF compared with TDF [6]. TFV is then primarily excreted in the urine [7, 8]. Prior research has demonstrated that urine TFV persists at detectable levels in the urine of people taking TAF [8, 9], but the degree to which urine TFV levels are comparable [8] or significantly reduced [9] among patients taking TAF vs TDF at standard doses remains unclear. The purpose of this study was to leverage a TAF directly observed therapy (DOT) study to (*i*) determine urine TFV levels among individuals taking 25 mg TAF within 24, 48, and 72 hours postdose, and (*ii*) compare those levels to urine TFV levels from a prior study of DOT 300 mg TDF.

METHODS

We leveraged urine specimens from The Cellular Pharmacology of F-TAF in Dried Blood Spots (TAF-DBS), a randomized prospective DOT study performed at the University of Colorado (ClinicalTrials.gov identifier NCT02962739) [10]. In this study, adults without HIV deemed at low risk for HIV infection were randomized to take TAF 25 mg/FTC 200 mg at either 33%, 67%, or 100% of daily dosing for 12 weeks. Participants assigned to the 100% dosing strategy took TAF/FTC each day while those assigned to 33% or 67% dosing schemes took either (*i*) 1 daily dose followed by 2 skipped days, or (*ii*) 2 daily doses followed by 1 skipped day, respectively, repeated over 12 weeks. TAF-DBS excluded patients with estimated glomerular filtration

Received 16 February 2021; editorial decision 12 April 2021; accepted 14 April 2021.

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Open Forum Infectious Diseases®2021

DOI: 10.1093/ofid/ofab200

rate <60 mL/minute/1.73 m^2. All TAF doses were directly observed in person, via livestream, or time-stamped video; study personnel recorded the date and time of each TAF dose. Urine specimens were collected after 4 and 8 weeks of TAF dosing at variable intervals 24–72 hours postdose (depending on timing of the urine collection visit), then stored at –80°C until testing.

To compare urine TFV levels on DOT TAF vs TDF, we also leveraged urine concentration data from the Tenofovir Adherence to Rapidly Guide and Evaluate PrEP and HIV Therapy (TARGET) trial, a previously reported randomized open-label DOT study of TDF in Thailand (ClinicalTrials.gov identifier NCT03012607) [11, 12]. In TARGET, healthy adult participants were randomized to take TDF 300 mg/FTC 200 mg either once daily (high adherence), 4 times per week (moderate adherence), or 2 times per week (low adherence) over a 6-week treatment period. TARGET participants were required to have an estimated glomerular filtration rate (eGFR) >60 mL/minute/1.73 m^2. All TDF/FTC doses were observed in person Monday–Friday; weekend doses were monitored by video/phone. Spot urine samples were collected throughout the study. These samples have been previously aliquoted and diluted to 1:1000 (to compare TFV levels with those reported in the literature and because TFV concentrates in the urine [3]), with urine TFV levels measured via LC-MS/MS using validated methods in the University of California, San Francisco (UCSF) Hair Analytical Laboratory (HAL).

In the DOT TAF study, for TAF-DBS participants receiving each of the 3 TAF dosing strategies, we similarly aliquoted urine samples, diluted to 1:1000, and quantified urine TFV levels via LC-MS/MS at the UCSF HAL. The lower limit of quantification (LLOQ) of the LC-MS/MS based assay was 40 ng/mL. Because time since the most recent dose determined urine TFV concentrations in both TARGET and a prior analysis using TAF [9], we present urine concentrations as time since last TFV dose, regardless of assigned dosing strategy.

To compare urine TFV concentrations for patients on DOT TAF vs TDF across all preassigned dosing strategies, we used a mixed-effects linear regression model with natural-log-transformed urine TFV levels measured by LC-MS/MS as the dependent variable and days since last TAF/TDF dose as the independent variable. This model was used to calculate the geometric mean ratio (GMR) comparing urine TFV levels on TAF vs TDF over 24, 48, and 72 hours.

Our study made use of urine samples previously collected from the participants of TARGET and TAF-DBS. All participants in both trials provided written informed consent. TARGET was approved by the ethics committees at the Institute for the Development of Human Research Protections at the Medical Sciences Department, Thai Ministry of Public Health; Sanpatong Hospital; Faculty of Associated Medical Sciences at Chiang Mai University; and the University of Washington Institutional Review Board in Seattle. TAF-DBS was approved by the Colorado Multiple Institutional Review Board.

RESULTS

Thirty-six TAF-DBS participants [10] (17 female, 7 black, and 6 Latinx, with a median age of 29 [range, 18–41] years and a median eGFR of 98 [range, 78–137] mL/minute/1.73 m^2) each provided 2 urine samples for this analysis (72 urine samples total).

The TARGET analysis included 28 individuals (12 female, all Asian) with a median age of 33 (interquartile range [IQR], 28–40) years and median eGFR of 108 (IQR, 94–119) mL/minute/1.73 m^2 [12]. Overall, 143 spot urine samples from 28 individual TARGET participants who had taken TDF/FTC within the preceding 72 hours were available for this comparison.

In mixed-effects linear regression modeling, the GMR urine TFV levels at 24, 48, and 72 hours post–TAF/FTC dosing were 1535 (95% confidence interval [CI], 1223–1926) ng/mL, 680 (95% CI, 529–875) ng/mL, and 302 (95% CI, 216–421) ng/mL, respectively, compared with 5860 (95% CI, 4486–7654) ng/mL, 2597 (95% CI, 2069–3261) ng/mL, and 1151 (95% CI, 888–1492) ng/mL post–TDF/FTC dosing in TARGET (Figure 1).

Urine TFV levels were significantly lower with TAF vs TDF (GMR, 0.26 [95% CI, .19–.37]; $P < .001$), corresponding to 74% lower urine TFV levels with DOT TAF vs TDF across all timepoints (95% CI, 63%–81%). Dosing patterns were not significantly associated with urine TFV levels for patients on TAF in models incorporating time since last TAF/FTC dose ($P = .52$).

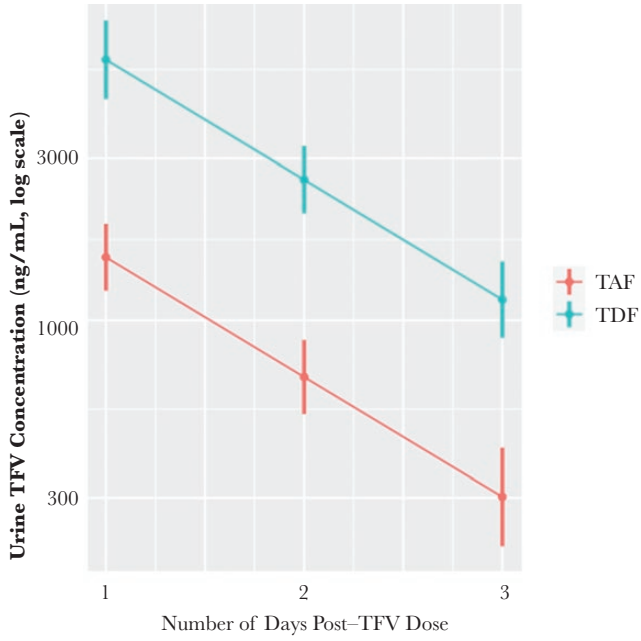

Figure 1. Urine tenofovir (TFV) concentrations among healthy participants taking tenofovir alafenamide (TAF) or tenofovir disoproxil fumarate (TDF) via directly observed therapy. Values represent point estimates and 95% confidence intervals.

DISCUSSION

Urine TFV levels for persons without HIV taking DOT TAF/FTC were 74% (95% CI, 63%–81%) lower than those seen in individuals taking DOT TDF/FTC across different timepoints postdose. This finding is similar to what is seen in the plasma, where TFV levels are approximately 90% lower with TAF than TDF [6]. The lower concentrations are likely because TAF is metabolized to TFV intracellularly in peripheral blood mononuclear cells while TDF is metabolized directly to TFV in the plasma and gut, leading to lower TFV equivalents in the plasma with TAF [7]. TFV is renally secreted and filtered, ultimately concentrating in the urine [7, 8], perhaps explaining why differences in urinary TFV concentrations for TAF vs TDF might be lower than the differences reported in plasma.

These results have 2 important implications for a forthcoming POC immunoassay to support TAF-based adherence monitoring. First, our results demonstrate that urine TFV levels are significantly lower with DOT TAF/FTC than TDF/FTC. Second, while urine TFV concentrations are lower with TAF than TDF, they remain detectable above the LLOQ of the immunoassay out to 72 hours post–TAF dose (following 1000-fold dilution), implying that the concentration cutoffs will remain within range to develop a lateral flow assay, as has already been accomplished for TDF [3–5]. The POC assay for TDF uses a cutoff of 1500 ng/mL to determine whether a person has taken TDF within the last 5 days. Based on participant feedback from earlier studies that poor specificity was distressing, this cutoff was selected to have high specificity (avoiding incorrect classification of people who had taken a TDF dose within the past 24 hours) as well as adequate sensitivity for nonadherence [4]. Our study demonstrates that GMR urine TFV levels with TAF approach this cutoff within just 24 hours of TAF dosing, leading to the potential for significant misclassification among individuals taking a TAF-based regimen every day as nonadherent. This implies that a separate POC assay with a lower TFV cutoff will be needed to assess adherence to either TAF-based antiretroviral therapy (ART) or PrEP. Such lateral flow assays allow for real-time feedback without the need for specialized laboratory equipment or personnel in routine clinical settings.

Strengths of our study include the ability to leverage 2 randomized trials of TAF and TDF—both of which incorporated strict DOT dosing strategies—to accurately assess and compare urine TFV concentrations among participants taking either TAF or TDF in the past 24, 48, or 72 hours. Limitations include the fact that all participants in the TARGET and TAF-DBS studies were healthy Thai and US volunteers considered at low risk for HIV acquisition, distinct from the eventual target populations of persons with diverse racial/ethnic backgrounds who are either living with HIV and on ART or vulnerable to HIV acquisition and on PrEP. A more general limitation for urine TFV immunoassays is that they serve as a short-term metric of adherence, confirming the presence or absence of recent TFV ingestion, without providing longer-term data as to patterns of adherence.

In conclusion, POC TFV immunoassays for TDF and eventually TAF could support efforts to rapidly identify patients with adherence challenges, improve patient/provider communication, inform clinical counseling messages, and support adherence interventions in real time across most commonly used HIV treatment and PrEP regimens. This work informs the development of a novel TFV urine immunoassay for TAF. As adherence remains a key challenge limiting the effectiveness of both daily PrEP and ART throughout the world, POC adherence support tools would be well positioned to not only improve patient-level care, but to support global efforts to end the HIV epidemic by "getting to zero" new HIV infections.

Notes

Acknowledgments. The authors thank the participants of the TAF-DBS and TARGET studies, without whom this work would not have been possible.

Financial support. This work was supported, in part, by the National Institute of Mental Health at the National Institutes of Health (NIH) (grant number K23MH122286 to M. S.); the National Institute of Allergy and Infectious Diseases, NIH (grant numbers R01AI152119 to M. G., R01 AI122298 to P. L. A., and R21 AI127200 to P. K. D.); and Gilead Sciences (grant number CO-US-412-399 to P. L. A.). This research was also made possible in part through support from the AIDS Research Institute at the University of California, San Francisco (Strategic Support award to K. A. J.).

Potential conflicts of interest. D. V. G. has accepted funds from Gilead Sciences. P. L. A. has received personal fees and research funding from Gilead Sciences. All other authors report no potential conflicts of interest. 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.

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