


Moderately High Tenofovir Diphosphate in Dried Blood Spots Indicates Drug Resistance in Viremic Persons Living with HIV

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Abstract

Background: Tenofovir diphosphate (TFV-DP) in dried blood spots (DBS) is a strong predictor of viral suppression in persons living with HIV (PLWH). Its association with antiretroviral therapy (ART) resistance remains unknown. **Methods:** Blood was collected in PLWH receiving TDF-containing ART enrolled in a 48-week study. Tenofovir diphosphate/emtricitabine triphosphate (FTC-TP) were quantified from the same sample as HIV viral load (VL) in PLWH who developed resistance within ≤ 12 months. **Results:** The study enrolled 807 participants, of whom 10 had new resistance-conferring mutations. Among these, median (interquartile range) TFV-DP and HIV VL were 956 (407-1510) fmol/punch and 9840 (513-68,200) copies/mL, respectively. Five had quantifiable FTC-TP in DBS. Based on previously published data, a TFV-DP concentration of 956 fmol/punch would have an adjusted odds of virologic suppression of 32.8 versus TFV-DP < 350 fmol/punch, making viremia of $\sim 10,000$ copies/mL an unexpected outcome. **Conclusion:** Moderately high TFV-DP in DBS (700-1249 fmol/punch) in PLWH with high viremia suggest that antiretroviral drug resistance might be present.

Keywords

ART, drug resistance, tenofovir diphosphate, emtricitabine triphosphate, dried blood spots

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Introduction

Adherence to antiretroviral therapy (ART) among people living with HIV (PLWH) is perhaps the most powerful predictor of virologic suppression and the prevention of ART resistance development.¹⁻⁵ The level of adherence necessary to achieve these outcomes varies within the literature,¹⁻⁴ and the lack of a gold standard for ART adherence quantification in PLWH has led to inconsistency in the adherence measures utilized in research and clinical practice.⁶ Due to the established association between adherence and virologic suppression,^{1,3} HIV viral load (VL) is often used in clinical practice as a surrogate marker for ART adherence. However, due to the potency of modern ART regimens, an undetectable VL may be achieved with adherence levels as low as 80% in some

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What Do We Already Know about This Topic?

In previously published research, we have established that tenofovir diphosphate (TFV-DP) is strongly associated with HIV viral suppression in persons living with HIV and that it predicts future viremia, even in patients who are virologically suppressed. We also have established that emtricitabine triphosphate (FTC-TP) in DBS is also associated with HIV viral suppression (beyond TFV-DP) and that is predictive of 3-day self-reported adherence.

How Does Your Research Contribute to the Field?

While the association of both of these adherence measures with HIV viral suppression has been clearly established, their association with antiretroviral drug resistance has not been evaluated, which was the focus of the current research.

What Are Your Research's Implications toward Theory, Practice, or Policy?

Our study is a step forward toward understanding the potential clinical applications of TFV-DP and FTC-TP in DBS as measures of adherence to ART. Our results suggest that TFV-DP in DBS could be used to identify a group of patients with viremia in whom antiretroviral drug resistance could be suspected.

patients.³ Therefore, relying on HIV VL alone cannot distinguish whether viremia is due to nonadherence versus the development of ART resistance. Thus, new strategies that can complement the adherence information provided by HIV VL in clinical practice are needed.

Tenofovir (TFV) undergoes intracellular phosphorylation to tenofovir diphosphate (TFV-DP) in peripheral blood mononuclear cells and red blood cells (RBCs). Tenofovir diphosphate is trapped within RBCs, leading to a long intracellular half-life of approximately 17 days.^{7,8} Tenofovir diphosphate in RBCs (measured using dried blood spots, DBS) is dose proportional, which makes it an informative measure of cumulative/averaged dosing over the preceding 8 weeks (ie, drug adherence) and exposure to TFV disoproxil fumarate (TDF) and TFV alafenamide.⁷⁻⁹ Similarly, emtricitabine (FTC) undergoes intracellular phosphorylation in RBCs to emtricitabine triphosphate (FTC-TP), with a half-life of approximately 1.5 days in this matrix, which translates into a measure of recent dosing (ie, within the previous 2-3 days).^{10,11} As an adherence assessment, TFV-DP in DBS has previously been shown to be predictive of efficacy to pre-exposure prophylaxis in high-risk men who have sex with men,¹² strongly associated with HIV viral suppression, and predictive of future viremia in PLWH.^{13,14} Similarly, FTC-TP

in DBS has also been associated with viral suppression, independently of TFV-DP.¹¹ Whether these adherence and exposure biomarkers are associated with ART drug resistance in those with viremia remains unknown. The objective of this study was to assess the relationship between TFV-DP and FTC-TP in DBS and the presence of resistance-associated mutations to antiretroviral medications in PLWH.

Methods

Herein we describe a subpopulation from a previously published parent study.¹³ Briefly, this was an observational, 48-week prospective study of PLWH, who were 18 years of age or older, receiving TDF-containing ART. The study was conducted at the University of Colorado Hospital Infectious Diseases Group Practice between June 2014 and July 2017. The study design consisted of up to 3 visits per participant within a 48-week time period which were separated by at least 2 weeks, as previously described.¹³

Cumulative ART exposure (adherence) was quantified by TFV-DP concentrations in DBS. Recent ART intake was also assessed by measuring FTC-TP in DBS. The procedure for quantification of both TFV-DP and FTC-TP from DBS has been previously described.^{8,13,15}

The primary outcome of the parent study was the association of TFV-DP with viral suppression, which has been reported elsewhere.¹³ The present subanalysis of this clinical cohort focused on the development of ART resistance in participants who had an HIV VL >20 copies/mL and paired DBS collection and in whom an HIV-1 genotype and/or phenotype were performed within 12 months of the last study visit. Resistance testing was obtained by the participant's provider in the usual course of clinical care without influence from the study team.

Participants were considered sensitive to their current regimen at baseline if they were ever able to achieve virologic suppression (<20 copies/mL) on their ART regimen or if they had a prestudy visit genotype and/or phenotype demonstrating sensitivity to all drugs included in their ART regimen. All mutations and/or resistance patterns identified on testing were recorded. Development of drug resistance was determined based upon changes from previous resistance testing, relationship of mutation/resistance patterns to current ART regimen, and temporal association with study visits and loss of virologic suppression. Development of resistance was defined as either (1) presence of a new resistance-associated mutation on post-visit genotype related to the participant's current ART regimen or (2) new resistance to a component of their ART regimen on post-visit phenotype. Resistance mutations were evaluated according to the IAS-USA 2017 Update of the Drug Resistance Mutations in HIV-1 and the Stanford University HIV Drug Resistance Database.¹⁶⁻¹⁸ Standard genotypes included reverse transcriptase and protease mutations, but not integrase, reflecting the availability of resistance testing at the time that this cohort was studied. They were performed at the University of Colorado Hospital and typically required a minimum VL of 1000 copies/mL. However, a genotype could have been run

at a lower VL via double extraction. All phenotype tests and genotype archive tests were performed externally. Phenotype tests required a minimum VL of 500 copies/mL.

All data were collected and stored in REDCap and Excel. Statistical analyses were performed using STATA and Microsoft Excel. Descriptive statistics were used for all study outcomes. Values are expressed as median (interquartile range [IQR]) or number (percent).

Ethical Approval and Informed Consent

Our study was approved by the Colorado Multiple Institutional Review Board (approval no. 13-2104). All participants provided written informed consent prior to enrollment for inclusion in the study, collection and use of blood samples, and clinical information. The parent study has been registered at clinicaltrials.gov (NCT02012621).

Results

Of the 807 patients included in the prospective parent study, DBS were assayed for TFV-DP in 532 participants. All participants (N = 174) who had at least 1 HIV VL >20 copies/mL at any of their study visits were included, as previously described.¹³ Within this population, a total of 23 participants had a genotype and/or phenotype obtained within 12 months of their last study visit. Of these, 10 had a genotype, 1 had a phenotype, and 12 had both a genotype and a phenotype available postvisit. Nine (90%) of 10 genotypes were standard genotype tests, and 1 was a genotype archive test.

Ten (43.5%) of the 23 participants in whom a genotype or phenotype was obtained developed new resistance mutations associated with their current ART regimen, which are the focus of our further description. The remaining 13 participants had no new resistance mutations associated with their ART regimen in the 12 months following their last study visit. All 10 participants with HIV resistance mutations were on TDF/FTC-containing regimens. The clinical and demographic characteristics of the study population are listed in Table 1.

Of the 10 participants who developed resistance to their ART, the most common mutations were M184V (n = 9, 90%), K65R (n = 2, 20%), and K70R (n = 2, 20%). Six (60%) participants who developed ART resistance had a post-visit phenotype available. Among these, resistance was most frequently documented to elvitegravir or raltegravir (n = 4, 67%) and FTC/lamivudine (n = 2, 33%).

The median (IQR) HIV VL at the time when the participants were first documented to be viremic was 9840 (513-68,200) copies/mL, and the median (IQR) TFV-DP concentration in DBS from the same blood draw was 956 (407-1510) fmol/punch. When averaging across all the available study visits, TFV-DP in DBS was 1055 (669-1579) fmol/punch and the corresponding HIV-RNA was 17,753 (6867-40,727) copies/mL. Five (50%) participants had quantifiable FTC-TP in DBS at the time when viremia was first documented. Of the 10 participants who developed resistance, 4 (40%) had

Table 1. Demographic and Clinical Characteristics among Participants with and without Documented ART Drug Resistance.

Variable	Developed Resistance (n = 10), Median (IQR) or n (%)	No Resistance Developed (n = 13), Median (IQR) or n (%)
Age (years)	44 (38-47)	46 (40-51)
Male	7 (70%)	12 (92%)
Race/ethnicity		
White ^a	7 (70%)	12 (92%)
Black	3 (30%)	2 (15%)
Other	0 (0%)	4 (31%)
Regimen type		
INSTI based	6 (60%)	3 (23%)
PI based ^b	2 (20%)	5 (39%)
NNRTI based	1 (10%)	2 (15%)
Mixed class	1 (10%)	3 (23%)
Single-tablet regimen	6 (60%)	4 (31%)
Duration on current therapy		
>3 and <6 months	2 (20%)	0 (0%)
>6 months	8 (80%)	13 (100%)
Time since diagnosis		
≥10 years	5 (50%)	10 (77%)
Treatment naive prior to current regimen	3 (30%)	3 (23%)
Resistance mutations prior to study ^c		
M184V	1 (10%)	4 (31%)
K103N/S	0 (0%)	3 (23%)
Time to genotype/phenotype (months)	0 (0-8)	4 (0-9)
Self-reported ART adherence (%) over the previous 3 months	83 (68-100)	88.5 (60-100)

Abbreviations: ART, antiretroviral therapy; INSTI, integrase strand transfer inhibitor; IQR, interquartile range; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor.

^aTwo participants who self-identified as white also identified as Hispanic or Latino.

^bAll PI-based regimens were boosted with either ritonavir or cobicistat.

^cThose without an available genotype or phenotype but who were virologically suppressed prior to study enrollment were considered to have no resistance mutations to their present regimen.

resistance testing, HIV VL, and DBS done on the same day. In contrast, the 13 viremic individuals who did not develop resistance had a median (IQR) TFV-DP concentration in DBS of 700 (362-1159) fmol/punch and HIV-RNA of 7640 (44-23,200) copies/mL at the first study visit with viremia, and 907 (794-1298) fmol/punch and 3640 (1460-30,647) copies/mL when averaging all study visits.

Discussion

In this study, we describe 10 cases of PLWH on TDF-based ART in whom moderately high cumulative ART adherence and exposure were documented with concomitantly high viremia and who eventually developed ART drug resistance. These characteristics were present, but to a lesser extent, in 13 viremic participants without resistance. A longer study follow-up or

more targeted resistance testing may have revealed resistance mutations in these individuals.

These findings highlight one of several potential clinical applications of these adherence biomarkers to inform about ART adherence and exposure beyond HIV VL. A TFV-DP concentration in DBS of 956 fmol/punch in the individuals with HIV resistance mutations is between 700 and 1249 fmol/punch, which had an adjusted odds of virologic suppression of 32.8 compared to very low TFV-DP concentrations (<350 fmol/punch).¹³ Furthermore, the HIV-RNA in those with resistance mutations was 9840 (513–68,200) copies/mL compared with 142 (38–1268) copies/mL in those with TFV-DP between 700 and 1249 fmol/punch in the parent study.¹³ Taken together, an HIV-RNA ~10,000 copies/mL among participants with a TFV-DP concentration of 700 to 1249 fmol/punch (when suppression would be expected) suggests resistance might be present. Comparatively, the quantification of FTC-TP in DBS in only 50% of the participants who developed resistance supports the idea that this marker of recent dosing may be less suggestive of resistance in viremic PLWH.

This study offers several strengths, including the use of a large cohort of PLWH in a real-world clinical setting. Additionally, use of TFV-DP and FTC-TP as novel biomarkers provided an objective measure of ART adherence and exposure. Among its limitations is the lack of prospective collection of genotype or phenotype in all viremic participants during the study, resulting in only 10 participants with documented resistance development within the established time frame. Another limitation is the focus on TFV-DP and FTC-TP as the sole pharmacologic measures of ART adherence, without pharmacological measures for the anchor drug (ie, PI or INSTI) among those on multiple-tablet regimens. Additionally, a more sensitive drug resistance assay may have detected additional resistance mutations compared with the commercial assays used in this study.

In summary, our findings suggest that in combination with HIV VL, TFV-DP in DBS may have value in identifying PLWH in whom ART resistance should be suspected. Future research should focus on the utility of TFV-DP in DBS to promptly identify and/or prevent the development of ART resistance in clinical practice, including its use as a surrogate for HIV genotyping in resource-limited settings.

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Declaration of Conflicting Interests

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