Tenofovir Diphosphate arising from TAF is quantifiable in Dried Blood Spots

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ABSTRACT

Background: Tenofovir diphosphate (TFV-DP) in red blood cells measured with dried blood spots (DBS) is a useful marker of cumulative tenofovir disoproxil fumarate (TDF) dosing, given its long half-life of approximately 17 days. However, studies have not assessed TFV-DP in DBS arising from tenofovir alafenamide (TAF). We aimed to determine whether TFV-DP can also be quantified in DBS during TAF therapy.

Methods: DBS were obtained from HIV-infected individuals on TAF-based therapy, using convenience sampling at various times post dose. Participants were either TDF-naïve or had been off any TDF-based therapy for at least 5 months. TFV-DP in DBS was measured using a validated LC-MS/MS method. The observed concentrations of TFV-DP in DBS from TAF were compared with the expected concentration of TFV-DP from TDF for daily dosing based on a previously published pharmacokinetic model. Data are presented as median (range).

Results: A total of 10 samples from 10 participants (9 White males; 1 Black female) were analyzed. Median age was 40 (26-62) years, and median hematocrit was 46 (41-55) percent. All had HIV-RNA <200 copies/mL. Eight participants were considered to be at steady-state (>8 weeks of therapy) with a median of 14 (9-21) weeks on TAF. The median concentration of TFV-DP in DBS was 224 (83-254) fmol/punch in these individuals. Two participants were pre-steady-state with 16 days of therapy and their values were 54 and 93 fmol/punch, consistent with a long half-life. Of the samples analyzed at expected steady-state, 7 were obtained from individuals taking co-formulated emtricitabine/cobicistat/elvitegravir/10 mg TAF; median TFV-DP was 231 (83-254) fmol/punch. One sample was from an individual taking emtricitabine/25mg TAF; TFV-DP was 216 fmol/punch. All participants had detectable concentrations of FTC-TP in DBS, reflecting recent dosing within the last 48 hours. TFV-DP values arising from TAF therapy were lower than from daily TDF dosing (median 1560 fmol/punch).

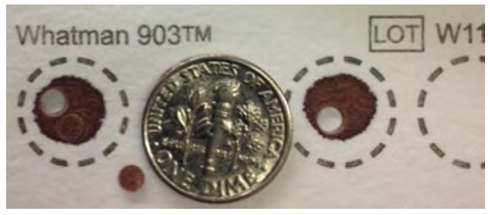
Conclusions: TFV-DP arising from TAF-based therapy is quantifiable in DBS, although at lower levels compared with TDF. TAF does not appear to load red blood cells with tenofovir to the same extent as peripheral blood mononuclear cells. Nevertheless, TFV-DP in DBS appears to be promising for use as a measure of cumulative dosing and adherence to TAF-based therapy. A directly observed therapy study is now underway to evaluate the pharmacokinetics and dose proportionality of TFV-DP arising from TAF.

BACKGROUND

- Tenofovir diphosphate (TFV-DP) has a 17-day half-life in red blood cells (RBCs) and can be measured in dried blood spots (DBS)[1]. This long half-life translates into an adherence gradient that is highly-informative of TFV dosing over a 2-3 month period of time and a strong predictor of pre-exposure prophylaxis (PrEP) efficacy[2].
- To date, TFV-DP in DBS has only been quantified during tenofovir disoproxil fumarate (TDF)-based therapy[1], and no data are available on the quantification of TFV-DP in DBS during tenofovir alafenamide (TAF)-based therapy.
- We aimed to determine whether TFV-DP can be quantified in DBS during TAF-based therapy.

METHODS

- HIV-infected individuals on a TAF-based regimen were enrolled at various time points after initiation of therapy during their regular clinic visit.
- All participants were either TAF/TDF naïve or had been off TDF for at least 5 months before initiation of TAF. Drug regimens evaluated included elvitegravir/cobicistat/emtricitabine/TAF 10 mg (E/C/F/TAF) or emtricitabine/TAF 25 mg (F/TAF).
- After venipuncture, 25 microliters of whole blood from EDTA tubes were spotted five times onto 903 Protein Saver Cards (Whatman/GE Healthcare, Piscataway, NJ) and allowed to dry for at least 2 hours (up to overnight). Samples were stored in plastic bags with humidity indicators at -80ºC until analysis.
- For extraction, a 3-mm diameter disk was punched from the DBS, micro-centrifuged using 500 mcl of 70:30 methanol-H_2O and sonicated for 10 minutes. TFV-DP and emtricitabine triphosphate (FTC-TP) were quantified using liquid chromatography tandem mass spectrometry (LC-MS/MS) as previously validated[3].
- The lower limit of quantification (LLOQ) for TFV-DP was 25 fmol/sample and for FTC-TP was 0.1 pmol/sample[4].
- The observed concentrations of TFV-DP from TAF were compared with the expected concentrations of TFV-DP from TDF based on a previously-published pharmacokinetic model[1].
- Data are presented as median (range).



RESULTS

- 10 HIV-infected individuals (9 White males; 1 Black female) were enrolled:
  - Median age 40 (20-62) years.
  - Median hematocrit 46 (41-55) percent.
  - All virologically-suppressed to <200 copies/mL at the time of visit.

Table. Tenofovir diphosphate levels in dried blood spots in HIV-infected individuals on TAF-based regimens.

Duration of therapy	Regimen	Weeks on TAF Median (range)	TFV-DP (DBS) fmol/punch Median (range)	TFV-DP (PBMC) fmol/10^6 cells Median (range)
>8 weeks	All (n=8)	14 (9-21)	224 (83-254)	488 (295-752)
	E/C/F/TAF 10 mg (n=7)	16 (9-21)	231 (83-254)	587 (333-752)
	F/TAF 25 mg (n=1)	9	216	295
<8 weeks	E/C/F/TAF 10 mg (n=2)	2	54, 93	445, 500

E/C/F/TAF: elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide 10 mg; F/TAF: emtricitabine/tenofovir alafenamide 25 mg; TFV-DP: tenofovir diphosphate.

- TFV-DP levels from TAF were approximately 85% lower than those expected from TDF with daily dosing (1560 fmol/punch)[1] and lower than levels in peripheral blood mononuclear cells (PBMCs).
- All individuals had quantifiable concentrations of FTC-TP in DBS, which is consistent with dosing within last 48 hrs[4].

CONCLUSIONS

- TFV-DP can be quantified in DBS from individuals on TAF-based regimens. However, levels are significantly lower when compared to TFV-DP in individuals on TDF-based regimens.
- In comparison to the high levels of TFV-DP from TAF observed in PBMCs, TAF does not appear to load RBCs to the same extent. This could be explained by the lower plasma concentrations of TFV observed during TAF-based therapy and/or by enzymatic differences in the intracellular metabolism of TAF between these two cell types[5].
- Despite the lower levels of TFV-DP observed during TAF-based therapy, TFV-DP in DBS is a promising biomarker of cumulative dosing and adherence to TAF. As an alternative to the 3-mm punch, larger spot areas could be extracted.
- A study evaluating the pharmacokinetics and dose proportionality of TFV-DP in DBS derived from TAF is underway (NCT02962739).

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