DUMOND JB ET AL: SUPPLEMENTAL MATERIALS

FULL DESCRIPTION OF METHODS

Clinical Study Design

Men receiving either TDF/FTC or TAF/FTC were recruited from the UNC Healthcare Infectious Diseases clinic. The protocol was approved by the UNC Biomedical Institution Review Board, and all participants provided written informed consent consistent with the principles of the Declaration of Helsinki prior to study procedure. Men were recruited into three study arms: HIV-positive receiving TDF/FTC (with appropriate background regimen), HIV-positive receiving TAF/FTC (with appropriate background regimen), and HIV-negative receiving TDF/FTC for HIV PrEP. HIV-infected men were included if their most recent viral load was undetectable; men receiving PrEP were included with confirmed negative HIV serology results. All men had at least 90% adherence by self-report, were free of sexually transmitted infections at screening, and agreed to abstain from sexual intercourse/masturbation for 48 hours prior to each study visit.

Paired blood samples and semen samples were obtained at 6 times post-dose (3, 6, 9, 12, 18, and 24 hours). Three samples were obtained within a 24-hour period; participants were offered the choice to schedule a single 48-hour visit or two separate 24-hour visits to the NC TraCS Clinical and Translational Research Center. Men were randomized to provide day 1 samples at either 3, 9, and 18 hours post-dose or 6, 12, and 24 hours post-dose. The men would then provide the alternative set of samples on day 2. An indwelling intravenous catheter was placed in a peripheral vein to obtain 14 mL of blood (6mL EDTA, 8mL CPT tubes, BD, Franklin Lakes, NJ, USA) per sampling point. Men self-collected semen samples by masturbation into a specimen cup. Men received their doses at their typical dosing time, with or without food as per the prescribing information of their regimen.

Sample Handling and Processing

EDTA tubes were kept on ice, while CPT tubes and semen samples were stored at room temperature until processing. EDTA tubes were centrifuged at 3000 g for 10 minutes, then blood

plasma (BP) was removed and stored in aliquots at -80C until analysis. CPT tubes were processed to recover peripheral blood mononuclear cells (PBMCs) as previously reported,(1) and counted on a Muse automated cell counter (Millipore Sigma, Darmstadt, Germany). Semen was processed as previously reported within 4 hours of collection to recover seminal plasma (SP) and seminal mononuclear cells (SMCs).(2) Specimen volume was recorded and SP was stored in 0.5 mL aliquots at -80C. SMCs were counted manually using a hemocytometer and stored as dry pellets at -80C to facilitate sample pooling, as needed.

Analytical Chemistry

TFV and FTC in BP and SP were measured as previously reported.(3) Parent TAF was also measured in BP and SP by LC-MS/MS, using a research-only, partially validated analytical method. TAF concentrations were measured with ±25% precision and accuracy. Both matrices were analyzed for TAF following protein precipitation extractions with internal standard tenofovir alafenamide-d5 by reverse phase chromatography (calibration range = 0.100-100ng/mL). TFV diphosphate (TFVdp), FTC triphosphate (FTCtp), and the endogenous nucleotides dATP and dCTP were measured in PBMCs and SMCs using previously described methods.(1) In order to increase sensitivity, SMC samples with <300,000 cell/mL were pooled within a participant. Based on the sensitivity of the assay (0.02 ng/mL), the sample volume, and the cell count, a sample-specific lower limit of quantification (LLOQ) was calculated.

BP and SP Virology

HIV RNA in BP and SP was measured using the Abbott RealTime HIV-1 Viral Load Assay (Abbott Laboratories. Abbott Park, Illinois, US). SP was diluted 1:1 with HIV-uninfected BP and then run using the standard BP protocol, accounting for the dilution in final HIV RNA value. The LLOQ was 40 copies/mL for BP and 80 copies/mL for SP.

SUPPLEMENTAL TABLE 1

Tenofovir (TFV) and Emtricitabine (FTC) Area Under the Curve (AUC) in Blood Plasma (BP) and Seminal Plasma (SP), and the SP:BP AUC Ratio, by Dosage Form and HIV Serostatus. TDF: tenofovir disoproxil fumarate; TAF: tenofovir alafenamide.

	TFV BP AUC (ng*hr/mL)	**TFV SP AUC (ng*hr/mL)**	**TFV SP:BP AUC Ratio**	**FTC BP AUC (ng*hr/mL)**	**FTC SP AUC (ng*hr/mL)**	**FTC SP:BP AUC Ratio**
HIV- TDF/ FTC	2797* (1579, 3558)	2702 (1803, 5320)	1.15† (0.75, 2.49)	9103 (6416, 10 745)	23 413 (17 409, 34 531)	2.88 (2.64, 3.06)
HIV+ TDF/ FTC	2635** (2133, 3149)	4124 (2539, 9044)	1.57‡ (0.85, 3.67)	7520 (6928, 8468)	32 208 (25 706, 36 263)	3.92 (3.62, 4.63)
HIV+ TAF/ FTC	318 (293, 353)	2384 (1791, 4318)	7.44 (5.27, 11.7)	10 441 (9425, 12 787)	39 997 (26 526, 50 939)	3.40 (2.48, 5.38)

* p=0.001 for comparison to HIV+ TAF/FTC

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† p=0.006 for comparison to HIV+ TAF/FTC

‡ p=0.029 for comparison to HIV+ TAF/FTC

SUPPLEMENTAL MATERIALS: REFERENCES

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2. Dumond JB, Reddy YS, Troiani L, Rodriguez JF, Bridges AS, Fiscus SA, et al. Differential extracellular and intracellular concentrations of zidovudine and lamivudine in semen and plasma of HIV-1-infected men. Journal of acquired immune deficiency syndromes (1999). 2008;48(2):156-62.

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