

Antiviral Activity, Safety, and Pharmacokinetics/Pharmacodynamics of Tenofovir Alafenamide as 10-Day Monotherapy in HIV-1–Positive Adults

Peter J. Ruane, MB, MRCPI,* Edwin DeJesus, MD,† Daniel Berger, MD,‡ Martin Markowitz, MD,§ U. Fritz Bredeek, MD,|| Christian Callebaut, PhD,¶ Lijie Zhong, PhD,¶ Srin Ramanathan, PhD,¶ Martin S. Rhee, MD,¶ Marshall W. Fordyce, MD,¶ and Kitty Yale, BS¶

Objective: To evaluate the antiviral activity, safety, pharmacokinetics, and pharmacokinetics/pharmacodynamics of short-term monotherapy with tenofovir alafenamide (TAF), a next-generation tenofovir (TFV) prodrug.

Design: A phase 1b, randomized, partially blinded, active- and placebo-controlled, dose-ranging study.

Methods: Treatment-naïve and experienced HIV-1–positive adults currently off antiretroviral therapy were randomized to receive 8, 25, or 40 mg TAF, 300 mg tenofovir disoproxil fumarate (TDF), or placebo, each once daily for 10 days.

Results: Thirty-eight subjects were enrolled. Baseline characteristics were similar across dose groups. Significant reductions in plasma HIV-1 RNA from baseline to day 11 were observed for all TAF dose groups compared with placebo ($P < 0.01$), with a median decrease of 1.08–1.73 $\log_{10}$ copies per milliliter, including a dose–response relationship for viral load decrease up to 25 mg. At steady state, 8, 25, and 40 mg TAF yielded mean TFV plasma exposures [area under the plasma concentration–time curve (AUC_{tau})] of 97%, 86%, and 79% lower, respectively, as compared with the TFV exposures observed with 300 mg TDF. For 25 and 40 mg TAF, the mean intracellular peripheral blood mononuclear cell tenofovir diphosphate AUC_{tau} was ~7-fold and ~25-fold higher, relative to 300 mg TDF.

Conclusions: Compared with 300 mg TDF, TAF demonstrated more potent antiviral activity, higher peripheral blood mononuclear cell intracellular tenofovir diphosphate levels, and lower plasma TFV exposures, at approximately 1/10th of the dose. This may translate into greater antiviral efficacy, a higher barrier to resistance, and an improved safety profile relative to TDF, supporting further investigation of TAF dosed once daily in HIV-infected patients.

Key Words: antiretroviral therapy, reverse transcriptase inhibitors, dose–response, pharmacodynamics, pharmacokinetics

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From the *Peter J. Ruane MD Inc, Los Angeles, CA; †Orlando Immunology Center, Orlando, FL; ‡Northstar Healthcare, Chicago, IL; §Aaron Diamond AIDS Research Center, The Rockefeller University, New York, NY; ||Metropolis Medical, San Francisco, CA; and ¶Clinical Research, Gilead Sciences, Foster City, CA.

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Correspondence to: Peter J. Ruane, MD, Peter J. Ruane MD Inc, 5901 W Olympic Blvd, Ste 401, Los Angeles, CA 90036 (e-mail: pjrune@lightsourcemedical.com).

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INTRODUCTION

The success of antiretroviral therapy (ART) has shifted clinical attention toward antiretroviral drug regimens that optimize tolerability, long-term safety, and durable efficacy. Where patients have access to treatment, morbidity and mortality are increasingly driven by non–AIDS-associated comorbidities, which are observed earlier than in age-matched controls, despite the best available ART.^{1,2} Likewise, patients are often diagnosed earlier, initiate therapy earlier, and look toward lifelong therapy, often greater than 50 years.³ The contribution of specific antiretroviral agents to long-term morbidity and mortality is increasingly important in this context. In regimens of comparable efficacy, the total pill burden, dosing frequency, and concerns about safety and side effects are significant obstacles to achieving high adherence.^{4,5} Current Department of Health and Human Services guidelines suggest tenofovir disoproxil fumarate (TDF) as a preferred

component of the nucleoside/nucleotide reverse transcriptase inhibitor [N(t)RTIs] backbone for treatment-naïve HIV-positive patients but that it is associated with nephrotoxicity and reduced bone mineral density.^{6,7}

Tenofovir alafenamide (TAF, formerly GS-7340) is an investigational, next-generation tenofovir (TFV) prodrug, which has a distinct metabolism designed to maximize antiviral potency and clinical safety. Unlike TDF, TAF is more stable in plasma and is predominantly hydrolyzed to TFV intracellularly by cathepsin A.⁸ This results in higher intracellular levels of the active phosphorylated moiety tenofovir diphosphate (TFV-DP) and lower circulating levels of TFV, relative to TDF.⁹ A radiolabeled distribution study in dogs demonstrated that, on a dose-per-dose basis, TAF administration leads to an increased distribution of TFV to tissues of lymphatic origin compared with TDF.¹⁰ Because TFV is actively transported from the blood into renal proximal tubule cells by the organic anion transporters OAT1 and OAT3, a reduction in plasma exposures of TFV may result in lower concentrations in proximal tubule cells and less nephrotoxicity.¹¹ Thus, an optimized dose of TAF could result in improved clinical efficacy and long-term safety relative to TDF.

Previously reported results from a 14-day monotherapy study (GS-120-1101) of TAF at 40 and 120 mg demonstrated significantly greater decreases in HIV-1 RNA, lower plasma TFV concentrations, and higher intracellular TFV-DP levels in peripheral blood mononuclear cells (PBMCs), compared with 300 mg TDF.⁹ The objective of this study, GS-US-120-0104, was to evaluate the antiretroviral activity, safety, pharmacokinetics (PK), and pharmacokinetic/pharmacodynamics of a lower range of TAF doses (8, 25, and 40 mg) as short-term monotherapy in HIV-1-infected patients.

MATERIALS AND METHODS

Subjects

ART-naïve and experienced HIV-1-positive adults at least 18 and less than 66 years of age, with no genotypic resistance to TFV [defined by the presence of K65R, Q151M, or T69 insertions or 2 or more of the following thymidine analog-associated mutations (TAMs): M41L, D67N, K70R, L210W, T215Y/F, or K219Q/E/N/R], with a CD4 cell count of 200 cells per microliter or more, and 2000 copies per milliliter plasma HIV-1 RNA or more, were eligible for enrollment. Patients were excluded if they had received any approved or investigational antiretroviral agent within 90 days before the first study dose, coinfection with hepatitis B or hepatitis C, or evidence of chronic liver disease. This study was conducted in accordance with Good Clinical Practice procedures, all applicable regulatory requirements, and the guiding principles of the Declaration of Helsinki. The study protocol was reviewed and approved by an institutional review board. All patients provided written informed consent before study entry.

Study Design

This was a phase 1b, multicenter, randomized, partially blinded, placebo- and active-controlled, dose-ranging study of TAF monotherapy for 10 days. Subjects were randomized

(2:2:2:1:2) to receive 1 of 3 doses of TAF (8, 25, or 40 mg), 300 mg TDF, or placebo every 24 hours for 10 days. All treatments were blinded except 300 mg TDF. Subjects were followed for 11 days after the end of dosing (to day 21). All study medications were administered orally in the morning in the fasted state with 240 mL of water. Observed study drug dosing was required at days 1–4 and days 7 and 10. Study drugs were dispensed to subjects after the day 4 clinic visit. For non-observed doses, subjects were instructed to take study drug daily in the morning at the same time each day in a fasted state. Dosing diaries were completed for all non-observed doses.

Antiviral Activity Assessments

The primary efficacy end point was the time-weighted average change from baseline to study day 11 (DAVG up to day 11) for plasma HIV-1 RNA. DAVG was computed using the same method as previously reported,¹² except that time was expressed in days rather than weeks. Samples for HIV-1 RNA were collected at screening, day 0 (baseline), and days 1, 2, 4, 7, 10, 11, 14, and 21. Plasma HIV-1 RNA was determined with the COBAS Amplicor HIV-1 Monitor Test version 1.5, ultrasensitive preparation (Roche Diagnostics, Branchburg, NJ) with a lower limit of detection of 50 HIV-1 RNA copies per milliliter. Whole venous blood samples were obtained on days 1 and 10 to analyze viral genotype using the GenoSureMG assay (Monogram Biosciences Inc, South San Francisco, CA). An allele-specific polymerase chain reaction (AS-PCR) assay was used to detect low-level mutations K65R and K70E in reverse transcriptase in plasma HIV-1 samples with >1000 copies per milliliter of HIV-1 RNA as previously described.¹³

Safety Assessments

After screening and day 0 (baseline), subjects were required to visit the clinic on days 1–4 and days 7, 10, 11, 14, and 21. Safety evaluations were completed as follows: adverse events (AEs) and concomitant medications on days 1–4, 7–11, 14, and 21; physical examinations at screening and days 0, 2, 7, 10, 14, and 21; fasting laboratory parameters on days 0, 7, 10, 14, and 21; and electrocardiograms at screening and days 0, 1, 4, 10, and 14.

Pharmacokinetic Assessments

Serial blood samples were collected for plasma and PBMC PK on days 1 and 10 at the following time points relative to study drug dosing: PK plasma: 0 (predose), 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 hours; PBMC: 0 (predose), 1, 2, 6, and 12 hours. Additional trough plasma samples were collected on days 2, 4, 7, 11, 14, and 21. Additional trough PBMC samples were collected on days 2, 4, 7, 11, 14, and 21 and at 0 (predose), 1, 2, 6, and 12 hours. Plasma samples were analyzed using a validated, high-performance liquid chromatography–tandem mass spectrometry method to determine TAF and TFV concentrations at QPS laboratories (Newark, DE). Pharmacokinetic parameters [including peak observed plasma concentration (C_{max}), time of

occurrence of $C_{\max}$ ($t_{\max}$), area under the plasma concentration–time curve during 1 dosing interval (AUC_{τ}), terminal elimination phase half-life ($t_{1/2}$), and concentration at the end of the dosing interval (C_{trough}) were estimated based on the observed concentration–time data by the noncompartmental pharmacokinetic approach using WinNonlin version 6.2 (Pharsight Corporation, Mountain View, CA). Pharmacokinetic and pharmacodynamic relationships were explored using an $E_{\max}$ model, where $\text{Effect} = (E_{\max} \times \log_{10} \text{PK parameter}) / (\log_{10} \text{PK parameter} 50 + \log_{10} \text{PK parameter})$. Day 10 $C_{\max}$ and AUC and changes in HIV-1 RNA were fit to $E_{\max}$ models that investigated both fixed (hill = 1) and variable hill slopes. Models were compared using goodness of fit to select the most appropriate model.

Statistical Analyses

A planned sample size of approximately 8 subjects per group in the 3 TAF treatment groups and 8 in the placebo-to-match TAF group was to provide 90% power to detect a treatment difference of 0.75 $\log_{10}$ copies per milliliter of DAVG11 in HIV-1 RNA between at least 1 of the 3 TAF treatment groups and the placebo group. In this power analysis, it was assumed that a common SD for DAVG11 in HIV-1 RNA was 0.27 $\log_{10}$ copies per milliliter (based on study GS-120-1101), and a 2-sided Wilcoxon rank sum test was conducted at an alpha level of 0.05. The time-weighted average change from baseline to study day 11 (DAVG up to day 11) for plasma HIV-1 RNA was summarized by treatment, and pairwise comparisons among treatment groups were performed using Wilcoxon rank sum test. The viral decay slope was fitted using log-linear regression model, where HIV-1 RNA collected on day 1 (the last available value before the first dose) and day 7 (the last available value up to day 7) were used as the dependent variable and day as the independent variable. The ratio of the mean slope between treatment pairs of interest was calculated. Comparison of the slopes from 2 different treatment

groups of interest was performed using Wilcoxon rank sum test. For computations, HIV-1 RNA values of less than 50 copies per milliliter were the assigned values of 49 copies. No adjustments for multiple comparisons were made. The relationships between pharmacokinetic parameters (AUC_{τ} and C_{τ}) and DAVG11 were assessed using Pearson correlation analysis. All statistical analyses were performed using SAS version 9.1 (SAS Institute Inc, Cary, NC).

RESULTS

Subjects

Of the 38 subjects (9 in 8 mg TAF, 8 in 25 mg TAF, 8 in 40 mg TAF, 6 in 300 mg TDF, and 7 in placebo) randomized and treated, demographics and baseline characteristics were similar (Table 1). Thirty-seven (97.4%) were men, 20 (52.6%) were White, and 14 (36.8%) were black. The mean age was 38 years (range: 20–57 years), the mean body mass index was 26.8 kg/m^2 (range: 19.9–37.3 kg/m^2), and the mean estimated glomerular filtration rate by Cockcroft-Gault was 118.2 mL/min (range: 64.2–173.9 mL/min). The mean CD4 count was 478 cells per microliter, and the mean baseline plasma HIV-1 RNA was 4.5 $\log_{10}$ copies per milliliter. At baseline, no subjects had resistance mutations to NRTIs or primary resistance mutations to protease inhibitors (PIs); 2 subjects had resistance mutations to nonnucleoside reverse transcriptase inhibitors (NNRTIs; 1 K103N and 1 V179D).

Antiviral Activity

The HIV-1 RNA responses for the 8-mg TAF treatment group were similar to that of the 300-mg TDF group. However, median DAVG₁₁ for plasma HIV-1 RNA ($\log_{10}$ copies/mL) in the 25- and 40-mg TAF treatment groups showed significantly greater decreases compared

TABLE 1. Demographics and Baseline Characteristics

Characteristic	TAF Once Daily					All (n = 38)
	8 mg (n = 9)	25 mg (n = 8)	40 mg (n = 8)	300 mg TDF (n = 6)	Placebo (n = 7)	
Men, N (%)	9 (100)	8 (100)	7 (88)	6 (100)	7 (100)	37 (97)
Race, N (%)						
Black	4 (44)	4 (50)	2 (25)	0	4 (57)	14 (37)
White	4 (44)	4 (50)	4 (50)	5 (83)	3 (43)	20 (53)
Mean age, years (minimum, maximum)	37 (20, 48)	41 (22, 57)	32 (28, 42)	41 (29, 52)	40 (27, 49)	38 (20, 57)
Plasma HIV-1 RNA, $\log_{10}$ copies per milliliter, mean $\pm$ SD (minimum, maximum)	4.5 $\pm$ 0.37 (3.8, 5.0)	4.5 $\pm$ 0.38 (3.7, 4.9)	4.3 $\pm$ 0.48 (3.4, 4.8)	5.0 $\pm$ 0.31 (4.6, 5.5)	4.2 $\pm$ 0.78 (2.8, 5.3)	4.5 $\pm$ 0.51 (2.8, 5.5)
Mean CD4 ⁺ cell count, cells per microliter (minimum, maximum)	535 (255, 904)	400 (278, 511)	502 (324, 784)	457 (148, 1131)	483 (275, 702)	478 (148, 1131)

with the 300-mg TDF treatment group (-0.94 and -1.08 vs -0.48 , $P = 0.017$ and $P = 0.006$, respectively). The median decrease in the plasma HIV-1 RNA levels from baseline to day 11 was also significantly greater for groups that received 25 mg TAF (-1.46 $\log_{10}$ copies/mL, $P = 0.024$) and 40 mg TAF (-1.73 $\log_{10}$ copies/mL, $P = 0.003$) compared with the group that received 300 mg TDF (-0.97 $\log_{10}$ copies/mL) (Fig. 1). Notably, TAF reaches near-maximal activity at a dose of 25 mg, whereas higher doses of TAF—including a comparison with doses of 40 and 120 mg from the previous study,⁹ GS-US-120-1101—do not show significant increases in HIV-1 RNA responses (Fig. 2). In patients receiving 25 and 40 mg TAF once daily, antiviral response was sustained between days 11 and 14 despite dosing discontinuation on day 10. The first-phase decay slopes for plasma HIV-1 RNA for the 25- and 40-mg TAF treatment groups were significantly steeper than for the 300-mg TDF treatment group ($P = 0.012$ and $P = 0.006$, respectively). Median first-phase decay slopes were -0.305 , -0.455 , -0.511 , and -0.183 /d for the 8-, 25-, and 40-mg TAF and 300-mg TDF treatment groups, respectively (Table 2).

Pharmacokinetics

All 25 subjects receiving TAF had detectable concentrations of TAF on day 1 after dosing commenced. After administration of 8, 25, or 40 mg TAF, TAF was rapidly absorbed (median T_{max} ~ 0.50 hours), displayed a short plasma half-life ($t_{1/2}$ ~ 0.40 hours), and was below the limit of quantitation in plasma by ~ 5 hours postdose. Accordingly, AUC_{last} was used as an exposure metric (vs AUC_{inf}). Given its short half-life, exposures were comparable between single- and multiple-dose administration (ie, no accumulation).

After administration of 8, 25, or 40 mg TAF or 300 mg TDF, the highest TFV plasma concentrations were observed with TDF. Multiple dosing resulted in higher TFV plasma concentrations vs single dose consistent with drug accumulation. Overall, TFV displayed linear PK consistent with its established profile. TFV exposures on day 10 after daily administration of 300 mg TDF yielded a mean (percentage of coefficient of variation) AUC_{tau} of 1918.0 (39.4) ng h/mL, consistent with historical data. Upon multiple dosing of 8, 25,

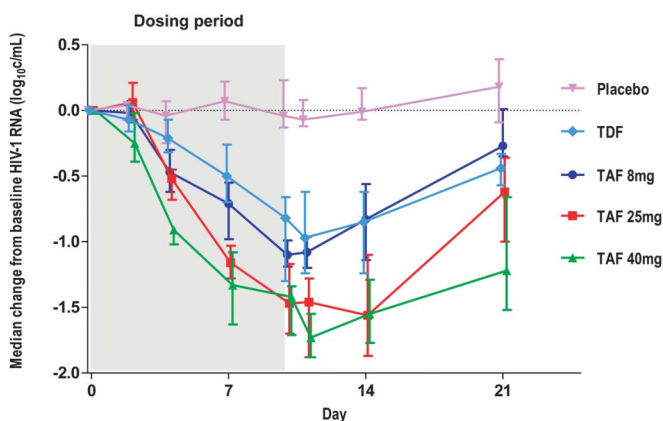


FIGURE 1. Median change from baseline in HIV-1 RNA.

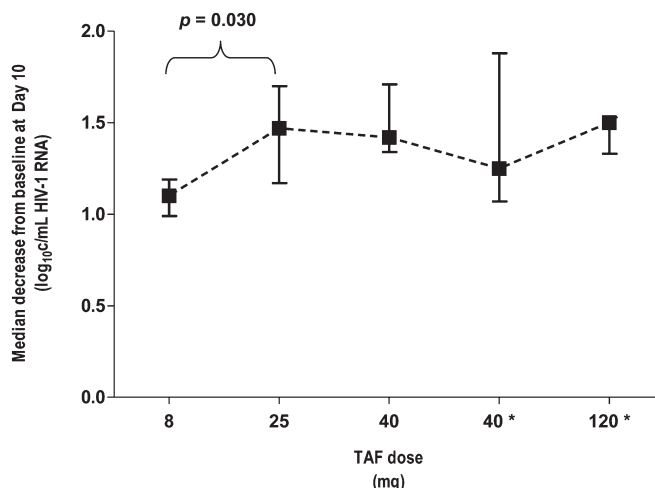


FIGURE 2. Median decrease from baseline HIV-1 RNA at day 10, by TAF dose. *, Data from GS-US-120-1101.

or 40 mg TAF, the mean TFV AUC_{tau} was 97%, 86%, and 79% lower, respectively, whereas mean TFV C_{max} was 98%, 94%, and 89% lower, respectively, as compared with 300 mg TDF (Table 3). TFV-DP concentrations in PBMCs (AUC) were comparable with 8 mg TAF and 300 mg TDF and markedly higher with 25 (~ 7 -fold) and 40 mg TAF (~ 25 -fold). On the last sampling day (day 21; 11 days after last dose), TFV-DP was quantifiable in 50% of subjects after 8 mg TAF, 100% of subjects after 25 and 40 mg TAF, and 15% of subjects dosed with 300 mg TDF.

Pharmacodynamics

Data from this proof-of-concept study demonstrate potent antiviral activity in HIV-1-infected patients. A median (minimum, maximum) change from baseline to day 11 in HIV-1 RNA of -1.08 (-1.57 , -0.18), -1.46 (-2.00 , -0.77), and -1.73 (-2.06 , -1.51) $\log_{10}$ copies per milliliter was observed after 8, 25, or 40 mg TAF treatment, respectively, as compared with a change of -0.97 (-1.30 , 0.24) $\log_{10}$ copies per milliliter with 300 mg TDF. Median viral load declines for both the 25- and 40-mg TAF doses were statistically greater than the 8-mg dose, but not significantly different from one another. TAF AUC fits well with an E_{max} model, with an E_{max} of ~ 1.7 to 1.8 $\log_{10}$ decline from baseline and EC_{50} for AUC of ~ 32 ng h/mL. A similar fit/ E_{max} estimate was also obtained using TAF C_{max} , which was somewhat expected given its relatively brief plasma circulation time frame driven by a short half-life and the resulting contribution of the C_{max} to the overall AUC. When compared with 40-mg and historical 120-mg data, 25 mg TAF approaches near-maximal activity. The correlation between TAF dose/plasma exposures and antiviral activity was consistent with its enhanced permeability (vs TFV) into PBMCs and in turn higher dose-dependent intracellular TFV-DP compared with TDF. As expected, plasma TFV exposures, which were substantially lower with TAF vs TDF, did not correlate with antiviral activity.

TABLE 2. Antiviral Activity

Characteristic	TAF Once Daily			300 mg TDF (n = 6)	Placebo (n = 7)
	8 mg (n = 9)	25 mg (n = 8)	40 mg (n = 8)		
Median DAVG11*, log ₁₀ copies per milliliter (minimum, maximum)	−0.76 (−0.97, −0.24)	−0.94 (−1.31, −0.54)	−1.08 (−1.46, −0.84)	−0.48 (−0.94, −0.11)	−0.01 (−0.08, 1.01)
P vs 300 mg TDF	0.216	0.017	0.010	—	0.038
Median Δ in HIV-1 RNA at day 11, log ₁₀ copies per milliliter (minimum, maximum)	−1.08 (−1.57, −0.18)	−1.46 (−2.00, −0.77)	−1.73 (−2.06, −1.51)	−0.97 (−1.30, 0.24)	−0.07 (−0.22, 0.08)
P vs 300 mg TDF	0.768	0.024	0.003	—	0.038
Median first-phase decay slope†, log ₁₀ copies per milliliter (minimum, maximum)	−0.305 (−0.525, −0.074)	−0.455 (−0.707, −0.201)	−0.511 (−0.708, −0.374)	−0.183 (−0.415, −0.001)	0.036 (−0.205, 0.412)
P vs 300 mg TDF	0.18	0.012	0.006	—	0.027

*Time-weighted average change from baseline up to day 11 (DAVG11) in plasma HIV-1 RNA (log₁₀ copies/mL).

†The viral decay slope was based on the HIV-1 RNA measurements on days 1 and 7 and between days 1 and 7.

Antiviral Resistance

All 38 randomized and treated subjects were analyzed for preexisting resistance in the PR and RT portions of the pol gene. As required by the enrollment criteria, all subjects had genotypic sensitivity to TFV at screening. No NRTI resistance mutations were observed at study entry. NNRTI resistance mutations were observed in 2 of 38 subjects (5.3%), specifically K103N and V179D in 1 subject each. Primary PI resistance mutations were not observed in any of the subjects. Postbaseline genotypic PR and RT data were available for the 37 of the 38 subjects (97.4%) at day 10. No subject developed any resistance mutations known to reduce susceptibility to TFV (K65R, Q151M, T69 insertions, or TAMs) nor any other NRTI, NNRTI, or PI resistance mutations by standard genotypic analyses. More sensitive genotypic analysis was

also performed on plasma samples to monitor for minor variants using an AS-PCR. This quantitative assay can detect low-level TFV mutations K65R and K70E down to 0.5% within an HIV-1 population (compared with ~25% using the standard sequencing). AS-PCR data were available at both baseline and day 10 time points for 87% (33/38) of the subjects. No subject from any treatment group (8, 25, and 40 mg TAF, 300 mg TDF, or placebo) had detectable K65R or K70E mutations down to the assay cut-off of 0.5% at either baseline or the end of 10 days of monotherapy treatment.

Safety

Through 10 days of monotherapy, TAF was generally well tolerated. The most frequently occurring treatment-emergent

TABLE 3. Steady State Pharmacokinetics on day 10

Parameter	TAF Once Daily			300 mg TDF (n = 6)
	8 mg (n = 9)	25 mg (n = 8)	40 mg (n = 8)	
TAF multiple dose PK day 10				
AUC _{last} (ng h/mL), mean (% CV)	54.7 (92.6)	115.2 (33.4)	308.9 (33.6)	—
C _{max} (ng/mL), mean (% CV)	85.8 (116.3)	223.6 (58.8)	629.5 (57.0)	—
T _{max} (h), median (Q1, Q3)	0.50 (0.50, 0.50)	0.50 (0.50, 0.75)	0.50 (0.38, 0.50)	—
t _{1/2} (h), median (Q1, Q3)	0.38 (0.26, 0.50)*	0.39 (0.34, 0.54)	0.42 (0.32, 0.49)	—
TFV multiple dose PK day 10				
AUC _{tau} (ng h/mL), mean (% CV)	65.5 (23.5)	267.7 (26.7)	405.8 (12.7)	1918.0 (39.4)
C _{max} (ng/mL), mean (% CV)	4.2 (24.7)	15.7 (22.1)	28.3 (8.7)	252.1 (36.6)
C _{tau} (ng/mL), mean (% CV)	2.1 (33.8)	9.2 (26.1)	13.3 (16.0)†	38.7 (44.7)
T _{max} (h), median (Q1, Q3)	1.50 (1.00, 1.98)	1.50 (1.25, 1.75)	1.29 (1.04, 1.50)	1.25 (0.58, 2.00)
t _{1/2} (h), median (Q1, Q3)	30.77 (26.90, 55.61)‡	40.19 (28.98, 44.84)	35.95 (26.38, 42.90)†	14.86 (12.18, 16.81)
PBMC TFV-DP multiple dose PK				
AUC _{tau} (μM h), mean (% CV)	3.5 (77.1)§	21.4 (76.9)	74.5 (92.7)¶	3.0 (119.6)#

AUC_{last} is presented for multiple dose PK because TAF concentrations are below the level of quantification by approximately 5 hours postdose and using AUC_{last} instead of AUC_{inf} or AUC_{tau}, respectively, provides a more appropriate measure of exposure assessment.

*n = 8.

†n = 7.

‡n = 8.

§n = 6.

||n = 4.

¶n = 7.

#n = 4.

% CV, percentage of coefficient of variation.

AEs in any treatment group in 2 or more subjects were nausea and fatigue: 2 of 8 subjects who received 40 mg TAF experienced nausea and 2 of 9 subjects who received 8 mg TAF experienced fatigue. The remaining AEs occurred in only 1 subject receiving study treatment. No drug-related AE occurred in more than 1 subject. No subject experienced a treatment-emergent AE that led to premature study drug discontinuation. Most treatment-emergent AEs experienced by subjects during the study were grade 1 (mild) or grade 2 (moderate) in severity. A grade 3 (severe) treatment-emergent AE was reported in 1 subject receiving placebo. No subjects experienced a grade 4 (life threatening) treatment-emergent AE during this study. One treatment-emergent serious AE was reported in a subject receiving 25 mg TAF (chest pain) but was not considered by the investigator to be related to study drug. No deaths or pregnancies occurred during this study. The majority of treatment-emergent laboratory abnormalities were grade 1 or grade 2. No treatment-emergent graded serum creatinine, serum phosphate, or urine glucose laboratory abnormalities were reported, and graded urine protein laboratory abnormalities were reported with similar frequency in each treatment group.

DISCUSSION

This proof-of-concept study demonstrated that a TAF dose as low as 25 mg had substantially reduced TFV exposures with improved pharmacodynamics compared with 300 mg TDF and substantially informs the clinical development of this compound. The antiviral dose–response relationship for TAF is now well characterized down to 8 mg (Fig. 2). At the 8-mg dose, the antiviral effect of TAF was similar to 300 mg TDF and predictably increased with higher doses of TAF. Notably, a TAF dose of 25 mg demonstrated a significantly greater antiviral effect than 8 mg TAF, whereas higher doses of TAF did not show a significant difference in this study of subjects without NRTI resistance mutations. Accompanying pharmacokinetic data demonstrate a dose-dependent increase in intracellular PBMC TFV-DP exposures, with mean TFV-DP AUC_{tau} values similar between 8 mg TAF and 300 mg TDF and ~7- and ~25-fold higher with 25 and 40 mg TAF, respectively.

Achieving higher intracellular TFV-DP exposures may have clinical use beyond the effect on plasma viral load, potentially including a higher barrier to the development of TFV resistance, activity against HIV-1 variants with mutations associated with TFV resistance, superior durability of combination regimens, and potential impact on low-level virus replication. Whereas viral resistance to TAF or TDF did not develop in this trial, further study would be required to determine if higher intracellular exposures of TFV-DP present a higher barrier to the development of resistance that is observed with 300 mg TDF. The increase in TFV-DP, the active antiretroviral moiety of TFV, would be expected to result in a similar increase in the intracellular inhibitory quotient, potentially leading to improved antiviral activity against viruses with mutations that confer moderate increases in TFV EC₅₀, such as K65R or multiple TAMs.

The precise characteristics of antiretroviral drugs that lead to long-term durability in the clinic are unknown but may be related to durable antiviral activity after drug dosing. From a patient's point of view, skipped doses, acute illnesses, and other clinical events that lead to suboptimal delivery of the active drug moiety to the site of HIV replication are likely to occur over long-term chronic therapy. In this respect, it is an encouraging observation that in this study, subjects who received 25 mg TAF once daily had an antiviral response that was sustained between days 11 and 14 despite dosing discontinuation on day 10. The parent drug, TFV, is already unique in the nucleos(t)ide analog class, because of its long half-life as the active diphosphate in uninfected cells and limited development of resistance *in vivo*.

Although the TAF dose of 25 mg resulted in significantly greater intracellular TFV-DP exposures and a greater antiviral effect, importantly, it also yielded systemic exposures of TFV that were substantially lower (~86%), with a mean AUC_{tau} of 267.7 ng h/mL compared with 1918.0 ng h/mL at steady state. TAF was rapidly absorbed with detectable levels by 0.25 hours postdose, with quantifiable levels in plasma for ~4 to 5 hours postdose. In comparison, TDF is not detectable at any time point postdose, providing clinical evidence in HIV-positive subjects for the observation that TAF is more stable than TDF in plasma.¹⁰

When interpreting the potential clinical use of these short-term data, the current standard prodrug of TFV, 300 mg TDF, serves as a useful reference because substantial clinical experience characterizes its antiviral effect as monotherapy¹⁴ and the durable efficacy and safety of TDF as part of multiple combination regimens for the long-term treatment of chronic HIV infection.¹⁵ How these observed differences in plasma and intracellular pharmacokinetics between commercially available 300 mg TDF and the investigational drug TAF might translate into long-term safety and efficacy remains to be seen. High-dose subcutaneous TFV administered to non-human primates causes proximal renal tubular dysfunction, and detailed analyses of this dose-related toxicity suggest a relationship between TFV clearance and/or AUC and renal and bone toxicities.¹⁶ Therefore, a prodrug that delivers substantially less plasma TFV exposures may reduce these known TFV-associated toxicities. It should be noted, however, that the higher intracellular levels of TFV-DP after administration of TAF open the possibility of safety issues not observed with TDF. In this study, TAF was well tolerated in each dosing arm and showed safety profiles similar to that of the 300-mg TDF treatment group.

HIV-positive patients today initiate therapy earlier and currently must look ahead toward uninterrupted ART for life, which is increasingly complicated by the morbidity and mortality associated with age-associated comorbidities. Although TDF-based regimens are commonly prescribed in the treatment of HIV infection, a next-generation TFV prodrug, optimized for long-term safety and tolerability, maintenance of viral suppression, and prevention of drug resistance, could bring substantial benefits to individual patients. Compared with 300 mg TDF, TAF, with doses as low as 25 mg, demonstrated greater antiviral effect at less than 1/10th the dose, with an almost 90% reduction in circulating TFV. These results support

a broad evaluation of this next-generation TFV prodrug in larger and longer term studies in HIV-1–positive patients. Clinical studies of TAF administered as a once-daily, single-tablet regimen with elvitegravir, cobicistat, and emtricitabine are currently underway.

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