Pharmacokinetics and renal safety of tenofovir alafenamide with boosted protease inhibitors and ledipasvir/sofosbuvir

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Background: Ledipasvir/sofosbuvir increases tenofovir plasma exposures by up to 98% with tenofovir disoproxil fumarate (TDF), and exposures are highest with boosted PIs. There are currently no data on the combined use of the newer tenofovir prodrug, tenofovir alafenamide (TAF), boosted PIs and ledipasvir/sofosbuvir.

Objectives: To compare the plasma and intracellular pharmacokinetics and renal safety of TAF with ledipasvir/sofosbuvir when co-administered with boosted PIs.

Methods: Persons with HIV between 18 and 70 years and on a boosted PI with TDF were eligible. The study was comprised of four phases: (1) TDF 300 mg with boosted PI; (2) TAF 25 mg with boosted PI; (3) TAF 25 mg with boosted PI and ledipasvir/sofosbuvir; and (4) TAF 25 mg with boosted PI. Pharmacokinetic sampling, urine bio-marker collection [urine protein (UPCR), retinol binding protein (RBP) and β_2 microglobulin (β_2 M) normalized to creatinine] and safety assessments occurred at the end of each phase. Plasma, PBMCs and dried blood spots were collected at each visit.

Results: Ten participants were enrolled. Plasma tenofovir exposures were 76% lower and tenofovir-diphosphate (TFV-DP) concentrations in PBMCs increased 9.9-fold following the switch to TAF. Neither of these measures significantly increased with ledipasvir/sofosbuvir co-administration, nor did TAF plasma concentrations. No significant changes in estimated glomerular filtration rate or UPCR occurred, but RBP:creatinine and β_2 M:creatinine improved following the switch to TAF.

Conclusions: Ledipasvir/sofosbuvir did not significantly increase plasma tenofovir or intracellular TFV-DP in PBMCs with TAF. These findings provide reassurance that the combination of TAF, boosted PIs and ledipasvir/sofosbuvir is safe in HIV/HCV-coinfected populations.

Introduction

Persons with HIV (PWH) are six times as likely to be coinfecting with HCV than those without HIV.¹ HCV coinfection is associated with a higher risk of liver-related morbidity and mortality in PWH,^{2,3} but despite these poor outcomes, some studies suggest uptake of HCV treatment is still suboptimal in this population.^{4,5} Several direct-acting antiviral therapies are now available for HCV treatment that have demonstrated similar cure rates between those living with and without HIV.^{6–9} However, PWH currently require lifelong therapy to suppress HIV replication, creating concern over potential drug–drug interactions between HIV and HCV treatment

regimens.^{10,11} Studies are needed to inform the safe and effective use of therapies for HIV and HCV infection when combined.

Ledipasvir and sofosbuvir are NS5A and NS5B inhibitors, respectively, that are available in a fixed dose combination (Harvoni[®], Gilead Sciences, Foster City, CA, USA) for HCV treatment. Ledipasvir/sofosbuvir is a globally recommended therapy for HCV and is administered over a period of 8–24 weeks depending on patient-specific characteristics.^{12,13} Cure rates over 95% have been achieved with this combination in PWH.^{6,14} Tenofovir is a key component of several recommended antiretroviral regimens for HIV treatment, and is available in the form of two different

prodrugs: tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide (TAF). TAF is the newer prodrug of tenofovir that has less renal and bone toxicity in comparison with TDF.^{15–17} This is because TAF is more stable than TDF in the blood, enabling greater cell loading, and results in ~90% lower plasma tenofovir and 2- to 7-fold higher tenofovir diphosphate (TFV-DP) exposures in PBMCs than those measured with TDF.^{16,18–20}

Although TDF is still widely used, it can cause proximal renal tubule injury and decreased bone mineral density, with higher tenofovir exposures corresponding to greater toxicity risk.^{21–23} Co-administration of ledipasvir/sofosbuvir with TDF increases plasma tenofovir exposures by 40%–98%,^{19,24} and TFV-DP concentrations in PBMCs and dried blood spots (DBS) by 2-fold and 17-fold, respectively.²⁵ Recent evidence suggests these increases are likely due to inhibition of carboxylesterase-mediated TDF hydrolysis by sofosbuvir^{26–28} and P-glycoprotein inhibition by ledipasvir.^{14,19} Boosted HIV PIs can also increase tenofovir exposures by ~22%–37% through inhibition of carboxylesterase and P-glycoprotein.^{12,29} Thus, the combined use of ledipasvir/sofosbuvir, boosted PIs and TDF results in tenofovir exposures that may exceed the range for which renal safety data are available.³⁰ Additional safety monitoring is recommended due to the increased risk of renal toxicity with this combination.^{31–33}

TDF and TAF are both prodrugs of tenofovir and are ultimately converted into the active TFV-DP form within cells, but their pharmacology differs. TAF 10 mg with elvitegravir/cobicistat resulted in slightly lower TAF exposures and 27% higher tenofovir exposures in plasma when co-administered with ledipasvir/sofosbuvir, which were still markedly lower than exposures measured with TDF, suggesting that no additional safety monitoring was required.³⁴ However, TAF is available in two different dosage strengths in the USA for use in PWH: 10 mg when co-formulated with cobicistat-containing fixed dose combinations (FDCs), such as darunavir/cobicistat/emtricitabine/TAF (Symtuza®, Gilead Sciences, Foster City, CA, USA), and 25 mg, which is co-formulated with emtricitabine (Descovy®, Gilead Sciences, Foster City, CA, USA) and other unboosted FDCs. Decisions between the 10 mg FDC versus 25 mg with a boosted PI are based on the patient’s treatment history and whether any PI-associated mutations necessitating twice-daily dosing are present.³² There are currently no pharmacokinetic or renal safety data for TAF at the standard 25 mg dose when administered with boosted PIs and ledipasvir/sofosbuvir.

Thus, the goal of this study was to compare the renal safety and plasma/intracellular pharmacokinetics of TDF, TAF, and TAF with ledipasvir/sofosbuvir in PWH on boosted PIs.

Patients and methods

Ethics

This study was approved by the Colorado Multiple Institutional Review Board (17-0490). All participants provided written informed consent. All study procedures were performed in accordance with the ethical standards of the University of Colorado Anschutz Medical Campus and with the Helsinki Declaration of 1975, as revised in 2000.

Study population

Persons living with HIV between 18 and 70 years of age were eligible. Participants were required to be on TDF with a boosted PI as part of clinical care at entry. Key exclusion criteria included an estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m², pregnant or breastfeeding women, medications not recommended for co-administration with TAF or ledipasvir/sofosbuvir, hepatitis B or chronic HCV infection, pathological fracture or other risk factors of bone loss or osteoporosis, unwillingness or inability to comply with study procedures, and any other uncontrolled medical conditions that would interfere with participation or interpretation of study outcomes in the opinion of investigators. Participants were enrolled starting in April 2018, and the last study visit took place in October 2019.

Study design

This was an open-label drug–drug interaction study conducted at the University of Colorado Anschutz Medical Campus (ClinicalTrials.gov identifier NCT03126370). The study design is outlined in Figure 1. Adherence was monitored throughout the study in real time using the Wisepill® RT2000 Medication Dispenser. Study visits were conducted at the end of each phase. At the Phase 1, 2 and 3 study visits, participants received a standardized meal (macronutrient-controlled 600 calorie meal consisting of 15% protein, 35% fat and 50% carbohydrates). Study medications, including other components of the participant’s antiretroviral regimen, were administered simultaneously under directly observed therapy after the standardized meal. Pharmacokinetic samples were collected at time 0 (pre-dose) and 1 and 4 h post-dose. DBS and PBMCs were isolated at time 0 using previously described methods.^{35,36} At the Phase 4 visit, a single convenience pharmacokinetic sample was collected. Safety laboratory assessments, including urine collections for assessment of renal biomarkers, were collected at each study visit. eGFR was calculated using the Chronic Kidney

Figure 1. The study was comprised of four phases: TDF 300 mg with a boosted PI for 12 weeks (Phase 1), TAF 25 mg with a boosted PI for 12 weeks (Phase 2), TAF 25 mg with a boosted PI and ledipasvir/sofosbuvir for 4 weeks (Phase 3), and TAF 25 mg with a boosted PI for 12 weeks (Phase 4). CBC, complete blood count; CMP, comprehensive metabolic panel; LDV/SOF, ledipasvir/sofosbuvir; /r, with ritonavir; /c, with cobicistat. This figure appears in colour in the online version and black and white in the printed version of JAC.

Disease-Epidemiology Collaboration (CKD-EPI) equation. Renal biomarkers included β_2 -microglobulin (β_2 M)-, retinol binding protein (RBP)- and urine protein-to-creatinine (Cr) ratios (UPCR). Adverse events were monitored and graded according to the Division of AIDS Adverse Event Grading Table version 2.1 (July 2017 version). Study data were collected and managed using REDCap electronic data capture tools at the Colorado Clinical & Translational Sciences Institute (CCTSI) with the Development and Informatics Service Center (DISC).^{37,38}

Bioanalytical methods

A reversed-phase UPLC-MS/MS assay for the determination of tenofovir, emtricitabine and TAF was developed and validated³⁹ for use with K₂ EDTA plasma matrix (see [Supplementary methods](#) at JAC Online). Intracellular TFV-DP in PBMCs and DBS were quantified using LC-MS/MS methods as previously described.^{40–42} DBS samples for TDF were quantified using both 3 mm and 2×7 mm punches and for TAF using 2×7 mm punches.

Pharmacokinetic analysis

Plasma tenofovir AUC over 24 h (AUC_{0–24}) for TDF was estimated using a two-compartment model with a first-order absorption rate and multiplicative error model (Phoenix[®] WinNonlin version 8.2, Certara[®], Princeton, NJ, USA). For this model, the sparse pharmacokinetic results with TDF from this study were pooled with tenofovir concentrations measured in a previously conducted intensive pharmacokinetic study in PWH.⁴³ A total of 360 observations from 40 individuals were pooled for analysis. Plasma tenofovir AUC_{0–24} with TAF was estimated by calculating steady-state concentrations for each participant and multiplying by 24 h, as the concentration versus time curves for tenofovir were relatively flat throughout the dosing interval with TAF.²⁰

Power and sample size estimates

The primary study outcomes were comparisons of plasma tenofovir AUC_{0–24} and intracellular TFV-DP concentrations in PBMCs between Phases 2 versus 1 and 3 versus 1. Sample size estimates for the primary outcomes were calculated using a two-sided Wilcoxon test with a significance level of 0.025. A tenofovir log_e-transformed mean (SD) of 8.321 (0.320) was previously measured in patients on TDF with ritonavir-boosted lopinavir.⁴³ Conservatively doubling the standard deviation, 10 participants provided >99% power to detect a 90% decrease between Phases 1 and 2,^{16,18} and an 87% decrease for Phase 3 versus 1, which assumed a 90% decrease from Phase 1 to 2 and 27% increase from Phase 2 to 3. For Phase 2 versus 1 PBMC comparisons, a geometric mean (CV) of 74 (48%) fmol/10⁶ cells was previously measured in individuals on TDF and a ritonavir-containing regimen,²⁸ with 7-fold higher concentrations expected with TAF.⁴⁴ Over 99% power was achieved to detect this magnitude of increase with conservatively doubling the standard deviation. An even greater increase in TFV-DP was expected in Phase 3 given ledipasvir/sofosbuvir increased tenofovir exposures by 27% in a prior study.³⁴

Statistical analysis

Pharmacokinetic parameters, eGFR and renal biomarkers were summarized using descriptive statistics. All outcomes were log_e-transformed prior to analysis with mixed models (SAS version 9.4). Study phase and adherence were examined as fixed effects, and participants were examined as random effects. Time since last dose was also examined as a fixed effect in PBMC models. Adherence was calculated as the number of openings divided by the number of days over the previous 1 month for PBMCs, and 3 months for DBS owing to the difference in half-lives and subsequent time to steady-state in each of these matrices. DBS results were compared using 2×7 mm punch sizes for TDF and TAF to compare levels using the same punch size. Results were back-transformed and comparisons between

phases were reported as the percentage change (97.5% CI) for the primary outcomes and $P < 0.025$ was considered statistically significant. For the secondary outcomes, 95% CIs and $P < 0.05$ was considered significant with no adjustments for multiple comparisons.

Results

Study population

Ten participants were enrolled and completed the study (9 male, 1 female; 1 black, 4 white, 5 Hispanic). At entry, mean (SD) age, weight and eGFR were 50 (12.3) years, 88.8 (16.6) kg and 91.5 (26.6) mL/min/1.73 m². Five participants were on darunavir/ritonavir, four participants were on darunavir/cobicistat and one participant was on atazanavir/ritonavir. All but one participant were suppressed (HIV viral load <200 copies/mL) at study entry. Median (range) 1 month adherence was 89% (65%–97%), 91% (72%–100%), 91% (78%–100%) and 86% (52%–100%) at the Phase 1, 2, 3 and 4 visits, respectively. Median (range) 3 month adherence was 90% (75%–99%), 90% (73%–100%), 91% (75%–98%) and 80% (67%–100%).

Pharmacokinetic results

Plasma tenofovir exposures decreased by 76% following the switch from TDF to TAF ($P < 0.0001$), and did not significantly increase with the addition of ledipasvir/sofosbuvir ($P = 0.08$) (Table 1 and Figure 2a). TAF plasma concentrations at 1 and 4 h post-dose did not significantly differ with ledipasvir/sofosbuvir (Table 1).

Adherence over the previous month and time since last dose were significant predictors of TFV-DP in PBMCs. After controlling for these factors, TFV-DP in PBMCs increased by 9.9-fold (95% CI 6.5, 15.1; $P < 0.0001$) after switching from TDF to TAF and by 11.4-fold (95% CI 8.0, 16.2; $P < 0.0001$) when comparing TAF with ledipasvir/sofosbuvir versus TDF (Table 1 and Figure 2b). TFV-DP in PBMCs did not significantly differ with the addition of ledipasvir/sofosbuvir in comparison with TAF with boosted PIs alone, or at the Phase 4 visit after stopping ledipasvir/sofosbuvir.

Adherence over the previous 3 months was a significant predictor of TFV-DP in DBS and subsequently controlled for in all DBS models. Geometric mean (CV) DBS concentration with TDF using the 3 mm punch size was 2507 (38.9%) fmol/punch. When comparing the same 2×7 mm punch size between TDF and TAF, TFV-DP concentrations in DBS were 81% lower with TAF ($P < 0.0001$) and 83% lower with ledipasvir/sofosbuvir versus TDF ($P < 0.0001$). TFV-DP concentrations were 11% lower with the addition of ledipasvir/sofosbuvir ($P = 0.011$), and 15% lower 3 months after stopping ledipasvir/sofosbuvir in comparison with TAF with boosted PIs in Phase 2 (95% CI 0.76, 0.95; $P = 0.01$) (Table 1 and Figure 2c).

Renal biomarker results

eGFR and UPCR did not significantly change following the switch from TDF to TAF or with the addition of ledipasvir/sofosbuvir (Table 2). Urinary β_2 M:Cr ratio decreased by 47% with the TDF to TAF switch (95% CI 0.30, 0.96; $P = 0.039$) and by 58% with the addition of ledipasvir/sofosbuvir (95% CI 0.21, 0.83; $P = 0.018$). Urinary RBP:Cr ratios decreased following the switch from TDF to TAF, although this did not reach statistical significance ($P = 0.092$). This decline was statistically significant following the addition of

Table 1. Plasma and intracellular pharmacokinetics of TDF and TAF across study phases

Outcome	TDF + b/PI (Phase 1)	TAF + b/PI (Phase 2)	TAF + b/PI + LDV/SOF (Phase 3)	Phase 2 vs 1	Phase 3 vs 1	Phase 3 vs 2
Plasma results ^a						
TFV AUC ₀₋₂₄ (ng·h/mL)	3466 (51.6%)	743 (35.8%)	868 (40.8%)	0.24 (0.14, 0.39) ^b <i>P</i> < 0.0001	0.28 (0.19, 0.40) ^b <i>P</i> < 0.0001	1.17 (0.98, 1.40) <i>P</i> = 0.08
TAF C _{1h} (ng/mL)	–	57.8 (68.2%)	50.9 (101%)	–	–	0.88 (0.44, 1.76) <i>P</i> = 0.69
TAF C _{4h} (ng/mL)	–	1.6 (134%)	2.0 (201%)	–	–	1.31 (0.59, 2.87) <i>P</i> = 0.47
Intracellular results ^a						
TFV-DP in PBMCs (fmol/10 ⁶ cells) ^c	83.0 (66.6%)	926 (23.4%)	1129 (34.9%)	9.9 (6.0, 16.3) ^b <i>P</i> < 0.0001	11.4 (7.5, 17.3) ^b <i>P</i> < 0.0001	1.15 (0.94, 1.40) <i>P</i> = 0.15
TFV-DP in DBS (fmol/punch) ^d	2507 (38.9%)	6735 (45.0%)	6100 (41.0%)	2.74 (2.09, 3.60) <i>P</i> < 0.0001	2.43 (1.86, 3.19) <i>P</i> < 0.0001	0.89 (0.82, 0.97) <i>P</i> = 0.011
TFV-DP in DBS (fmol/punch) ^e	36014 (46.4%)	6735 (45.0%)	6100 (41.0%)	0.19 (0.15, 0.25) <i>P</i> < 0.0001	0.17 (0.13, 0.22) <i>P</i> < 0.0001	0.89 (0.81, 0.97) <i>P</i> = 0.011

b/PI, boosted PI; C_{1h}, concentration at 1 h post-dose; C_{4h}, concentration at 4 h post-dose; LDV/SOF, ledipasvir/sofosbuvir.

^aPharmacokinetic outcomes summarized as geometric mean (CV) for each phase, and GMR (95% CI) unless otherwise indicated.

^bPrimary outcomes reported as GMR (97.5% CI).

^cPhase comparisons controlled for 1 month adherence and time since last dose.

^dTDF punch size of 3 mm versus TAF punch sizes of 2×7 mm; phase comparisons controlled for 3 month adherence.

^eTDF and TAF punch sizes of 2×7 mm; phase comparisons controlled for 3 month adherence.

ledipasvir/sofosbuvir to TAF in comparison with TDF [GMR 0.34 (95% CI 0.15, 0.75); *P* = 0.012] and TAF [GMR 0.60 (95% CI 0.37, 0.99); *P* = 0.047]. No significant changes in renal biomarkers occurred after stopping ledipasvir/sofosbuvir.

Adverse events

No serious adverse events (AEs), AEs requiring discontinuation of study medication, deaths or pregnancies occurred. Nearly all AEs deemed possibly or related were grade 1 (*n* = 15) or grade 2 (*n* = 4) in severity. Two separate grade 3 decreases in eGFR (based on absolute eGFR values of 30 to <60 mL/min/1.73 m²) occurred in the same participant while on TDF. One of these events was accompanied by a grade 1 serum Cr (SCr) increase. This participant had a grade 2 eGFR at study entry. Two other participants experienced grade 2 eGFR declines based on absolute values (one experienced two separate grade 2 decreases with TAF during Phase 2 and Phase 4, but was grade 2 at study entry; the other experienced one grade 2 decrease at the Phase 1 visit with TDF that later resolved). Another participant had a grade 1 SCr increase based on comparison with the upper limit of normal (ULN) (1.59 mg/dL reflecting 1.1–1.3 × ULN) at the Phase 3 visit (TAF and ledipasvir/sofosbuvir), but this value matched their SCr result at study entry.

Discussion

Plasma tenofovir exposures were 76% lower and TFV-DP concentrations in PBMCs were approximately 10-fold higher following the transition from TDF 300 mg to TAF 25 mg with a boosted PI. The addition of ledipasvir/sofosbuvir to TAF plus boosted PIs did not significantly increase plasma TAF, plasma tenofovir or TFV-DP in PBMCs. TFV-DP in DBS significantly decreased following the switch from TDF to TAF when comparing the same punch size between phases.⁴² The addition of ledipasvir/sofosbuvir did not increase TFV-DP in DBS. No significant changes in eGFR or UPCR were revealed, and improvements in urinary RBP:Cr and β2M:Cr ratios occurred following the switch to TAF. These markers also did not worsen with the addition of ledipasvir/sofosbuvir. These findings suggest the combination of boosted PIs with TAF and ledipasvir/sofosbuvir is likely to be a safe and effective combination in persons coinfecting with HIV and HCV requiring these therapies.

This study indicates that the impact of ledipasvir/sofosbuvir on the plasma and cellular pharmacokinetics of TAF is much less than the impact on TDF. Ledipasvir/sofosbuvir increases plasma tenofovir exposures with TDF by 40%–98%^{19,24} and increases TFV-DP concentrations in PBMCs and DBS by ~3-fold and ~7- to 18-fold, respectively.^{25,28} Conversely, studies with elvitegravir/cobicistat/TAF 10 mg/emtricitabine and ledipasvir/sofosbuvir revealed 14% lower TAF exposures and 27% higher tenofovir exposures in plasma.³⁴ Although tenofovir plasma exposures were increased,

Figure 2. Geometric mean (geometric SD) (a) plasma tenofovir exposures over 24 h, (b) TFV-DP concentrations in PBMCs and (c) TFV-DP concentrations in DBS (results with 2×7 mm punches for TDF and TAF) across study phases. This figure appears in colour in the online version of JAC and in black and white in the printed version of JAC.

total tenofovir exposures are much lower than those measured with TDF, suggesting that no additional safety monitoring is necessary. This was recently corroborated in a study of HIV/HCV-co-infected patients who were switched to elvitegravir/cobicistat/emtricitabine/TAF and treated with ledipasvir/sofosbuvir, which showed this combination was safe and effective.⁴⁵ The differences in drug–drug interaction magnitudes and safety profiles of TDF and TAF are driven by the differences in the pharmacology of these prodrug moieties. TDF is primarily hydrolysed by carboxylesterase type 2 (CES2) in the intestinal tract, whereas TAF is selectively hydrolysed by CES1 in the liver. Sofosbuvir is a more potent inhibitor

of CES2 than CES1,^{26,27} which results in greater delivery of TDF and its monoester intermediate to the portal blood and systemic circulation.²⁸ This in turn contributes to greater blood cell loading and significant increases in TFV-DP in PBMCs and DBS with TDF in comparison with TAF.

TAF is known to result in lower plasma tenofovir exposures and higher TFV-DP concentrations in PBMCs in comparison with TDF. The magnitude of decrease in plasma tenofovir levels between TDF and TAF in this study is similar but slightly less than those reported in previous studies, which showed ~90% lower levels.^{16,18} However, tenofovir AUCs measured with TDF⁴³ and TAF⁴⁶ in this study are comparable to those measured previously. These findings may be due to variable effects on plasma TAF and tenofovir levels with boosted PIs. In people without HIV, atazanavir/ritonavir increased plasma TAF AUC by 91%, whereas darunavir/ritonavir and darunavir/cobicistat did not alter TAF AUC.^{31,47} Although TAF changes vary, tenofovir AUC is increased 2.6- and 3.2-fold with co-administration of boosted atazanavir and darunavir, respectively.⁴⁶

The 9.9-fold increase in TFV-DP in PBMCs with the switch from TDF 300 mg to TAF 25 mg in this study is higher than the ~2- to 7-fold increases reported previously.^{16,18–20} This is likely due to use of a higher TAF dose (25 mg) and concomitant use of boosted PIs. Although the magnitude of increase is higher than was observed in previous switch studies, the measured TFV-DP concentrations are within the range of those measured during initial dose-finding studies with TAF.⁴⁸ The long-term clinical relevance of higher intracellular PBMC concentrations should be examined further. TFV-DP levels in PBMCs with TAF are lower with darunavir versus atazanavir, although both are still several-fold higher than those measured with TDF.⁴⁶ Only one participant in this study was on atazanavir, thus comparisons between PIs could not be performed.

DBS levels with TDF were comparable to levels measured in PWH on boosted PIs,⁴⁹ but TAF DBS levels were ~3-fold higher than those previously measured in people without HIV⁴² and PWH.⁴⁴ The decreases in DBS concentrations across Phases 2–4 were unexpected, but may be a function of carryover of TFV-DP from TDF and the differences in punch sizes that are assayed with each prodrug. TFV-DP in DBS with TAF is ~10-fold lower when comparing with the 3 mm punch size usually measured with TDF,⁴⁴ which may be due to preferential cell loading of TAF in PBMCs, lower tenofovir exposures, and differences in enzymatic conversion of TAF versus TDF in RBCs (i.e. lack of lysosomal cathepsin A in RBCs).⁴² Assuming a 17 day half-life, as has been measured in previous studies, this means that ~9%–34% of the TFV-DP concentrations measured 3 months after switching to TAF may be from residual TDF in this study. By 6 months, this estimated residual amount drops to ~3%–10%. Clinically, this means that DBS adherence interpretations with TAF should not be performed until at least 5–6 months after switching from a TDF-based regimen in PWH or persons on pre-exposure prophylaxis.

Urine RBP and β 2M have been used in clinical practice and various research studies to determine the presence or absence of proximal tubular dysfunction in PWH.^{50–52} Increases in these low molecular weight proteins are indicative of tubular dysfunction, and urinary concentrations correlate with the severity of the tubular dysfunction.⁵⁰ HIV infection alone can increase these measures,⁵³ and PWH with tenofovir-induced Fanconi syndrome tend to have very high levels of these markers.⁵⁴ Several studies

Table 2. Renal biomarkers across study phases

Outcome ^a	TDF + b/PI (Phase 1)	TAF + b/PI (Phase 2)	TAF + b/PI + LDV/SOF (Phase 3)	Phase 2 vs 1	Phase 3 vs 1	Phase 3 vs 2
eGFR (mL/min/1.73 m ²)	86.7 (27.6%)	91.0 (23.0%)	88.1 (24.9%)	1.05 (0.92, 1.20) <i>P</i> =0.43	1.02 (0.92, 1.12) <i>P</i> =0.71	0.97 (0.89, 1.05) <i>P</i> =0.40
UPCR (mg/g)	134 (65.7%)	118 (50.2%)	97.3 (41.0%)	0.89 (0.69, 1.14) <i>P</i> =0.31	0.73 (0.47, 1.12) <i>P</i> =0.14	0.82 (0.54, 1.26) <i>P</i> =0.34
β2M:Cr ratio (μg/g)	419 (176%)	224 (167%)	178 (156%)	0.53 (0.30, 0.96) <i>P</i> =0.039	0.42 (0.21, 0.83) <i>P</i> =0.018	0.79 (0.52, 1.22) <i>P</i> =0.25
RBP:Cr ratio (μg/g)	436 (174%)	242 (180%)	146 (91.6%)	0.56 (0.27, 1.12) <i>P</i> =0.09	0.34 (0.15, 0.75) <i>P</i> =0.012	0.60 (0.37, 0.99) <i>P</i> =0.047

b/PI, boosted PI; LDV/SOF, ledipasvir/sofosbuvir.

^aRenal safety outcomes summarized as geometric mean (CV) and GMR (95% CI).

with boosted PIs and TDF have revealed a link between this combination and higher risk of renal dysfunction, particularly with the use of atazanavir in comparison with darunavir.^{55,56} These effects have been attributed to higher tenofovir exposures, and pooled analyses have shown the improved renal safety profile of TAF in comparison with TDF.⁵⁷ Significant decreases in RBP:Cr and β2M:Cr ratios occurred following TDF-to-TAF switch in our study, which is in alignment with prior TAF switch studies. Ledipasvir/sofosbuvir has been associated with marked declines in eGFR among patients receiving regimens with and without TDF, longer treatment durations of 24 weeks, and cirrhosis.⁵⁸ Significant correlations between tenofovir AUC and elevated RBP:Cr and β2M:Cr ratios in patients receiving TDF with ledipasvir/sofosbuvir have also been identified.⁵⁹ In contrast to findings with TDF and ledipasvir/sofosbuvir, no significant changes in eGFR, UPCR or β2M:Cr ratio occurred with the combined use of TAF, boosted PIs and ledipasvir/sofosbuvir. Although a significant decrease in RBP:Cr did occur between Phases 2 and 3, this may be due to the continued improvement in urine biomarkers following the transition from TDF rather than a protective effect of ledipasvir/sofosbuvir.

Our participants had normal to mild-moderate impairments in renal function, and thus findings from this study cannot be directly extrapolated to those with advanced renal disease. Prior studies with TAF in patients with severe renal impairment⁶⁰ and on chronic haemodialysis⁶¹ have demonstrated a favourable safety profile. Both sofosbuvir and ledipasvir are also considered safe for use in patients with eGFR <30 mL/min/1.73 m² or on haemodialysis.⁶² TAF exposures with the 25 mg dose are ~79%–92% higher with severe renal impairment in comparison with those without renal disease, and tenofovir exposures are increased ~3- to 6-fold.⁶⁰ Though tenofovir exposures were higher, levels were still ~10%–40% lower than those measured with TDF 300 mg in persons with normal renal function. The addition of boosted PIs would likely further increase plasma tenofovir exposures with TAF in those with advanced renal disease, but the extent of additive increase with ledipasvir/sofosbuvir would likely be low to negligible.

There are limitations to this study. These findings were in a small sample size, but *a priori* sample size calculations demonstrated robust power to detect large differences between Phases 1 and 2 and Phases 1 and 3. A sparse sampling design was also used, and tenofovir plasma exposures with TDF were modelled using data from a previously conducted pharmacokinetic study as the base model.⁴³ However, the samples from the previous study were analysed in the same laboratory, participants had meals with similar fat content, and modelled tenofovir exposures were comparable to historical estimates. This study was not conducted in HIV/HCV-coinfected patients due to large-scale transition to TAF-containing regimens and coordinated efforts to treat this population. The Phase 3 duration was subsequently limited to 4 weeks, rather than 8–12 weeks. Though the full treatment was not administered, this duration of therapy still provided enough time to reach steady-state in plasma and PBMCs in the event of a drug–drug interaction. The half-life of TFV-DP in DBS is longer and thus steady-state was not achieved. However, previous studies with TDF and sofosbuvir still revealed marked increases in DBS at 4 weeks into therapy, which were not detected in this study.^{25,28}

Plasma tenofovir exposures were significantly lower and TFV-DP concentrations in PBMCs were significantly higher following TDF-to-TAF switch in PWH on boosted PIs. The combination of TAF with boosted PIs and ledipasvir/sofosbuvir did not significantly increase plasma or intracellular tenofovir levels. These pharmacokinetic findings were accompanied by improvements in markers of renal tubular dysfunction and a lack of change in eGFR and UPCR with concomitant use. Collectively, these results suggest this combination is safe and effective in treating HIV/HCV-co-infected populations without the need to modify their ART.

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Disclaimer

The contents are the authors' sole responsibility and do not necessarily represent the official views of the NIH or Gilead Sciences, Inc.

Supplementary data

Methods and Table S1 are available as [Supplementary data](#) at JAC Online.

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