



# Population Pharmacokinetic Modeling of Tenofovir in the Genital Tract of Male HIV-Infected Patients

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**ABSTRACT** The aims of this study were to describe the blood plasma (BP) and seminal plasma (SP) pharmacokinetics of tenofovir (TFV) in HIV-1-infected men, to assess the role of genetic polymorphism in the variability of TFV transfer into the male genital tract, and to evaluate the impact of TFV SP exposure on seminal plasma HIV load (spVL). Men from the Evarist-ANRS EP 49 study treated with TFV as part of their antiretroviral therapy were included in the study. A total of 248 and 217 TFV BP and SP concentrations from 129 men were available for the analysis. For pharmacogenetic assessment, a total of 121 single nucleotide polymorphisms (SNP) were genotyped. Data were analyzed using a nonlinear mixed-effects modeling approach. TFV pharmacokinetics were best described by a two-compartment model for BP and by an effect compartment with different input and output constants for SP. TFV exposures (area under the concentration-time curve from 0 to 24 h [AUC<sub>0-24</sub>]) were higher in SP than in BP (median AUC<sub>0-24</sub>, 7.01 versus 2.97 mg · liter<sup>-1</sup> · h, respectively). The median (range) SP-to-BP AUC<sub>0-24</sub> ratio was 2.24 (0.53 to 34.13). After correction for multiple testing, none of the SNPs were significantly associated with the TFV transfer rate constant. The impact of the TFV SP AUC<sub>0-24</sub> or TFV SP-to-BP AUC<sub>0-24</sub> ratio on spVL was not significant ( $P = 0.808$  and  $0.768$ , respectively). This is the first population model describing TFV pharmacokinetics in the male genital tract. TFV SP concentrations were higher than BP concentrations. Despite TFV SP exposures being higher than BP exposures, an spVL was detectable for 12.2% of the men.

**KEYWORDS** tenofovir, population pharmacokinetics, seminal plasma, genital tract

Sexual transmission of human immunodeficiency virus (HIV) is the main route of HIV infection spreading. The reduction of HIV replication in semen could play an important role in the sexual transmission of HIV. Using combined antiretroviral (ARV)

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**TABLE 1** Demographic and biological characteristics of the HIV-infected men in the study

Parameter <sup>a</sup>	Median (range)
Age (yr)	43 (27–63)
BW (kg)	73 (46–108)
S <sub>CR</sub> (μmol · liter <sup>-1</sup> )	78 (28–113)
CL <sub>CR</sub> (ml · min <sup>-1</sup> )	113 (67–368)

<sup>a</sup>Abbreviations: BW, body weight; S<sub>CR</sub>, serum creatinine; CL<sub>CR</sub>, creatinine clearance.

treatments (cART), some studies have reported a parallel decrease in HIV RNA levels in blood plasma (BP) and seminal plasma (SP) (1–3). However, other studies have described a discordance between HIV BP viral load and SP HIV RNA load (spVL) (4–6). Thus, despite suppression of HIV replication in BP reflected by undetectable HIV RNA levels, spVL was detectable for some patients receiving cART (7). This discordance suggests a compartmentalization of HIV replication in the male genital tract, where the distribution of antiretroviral drugs could be a determinant of HIV shedding (7). A poor drug distribution in this compartment could lead to suboptimal concentrations, allowing HIV replication. The distribution of antiretroviral drugs in the genital tract has been reported to be drug specific and highly variable among individuals even for the same drug (7–9). For some protease inhibitors, SP concentrations have been reported to be lower than BP concentrations, whereas indinavir SP concentrations have been reported to be much higher (9). For nonnucleoside reverse transcriptase inhibitors, nevirapine and efavirenz SP-to-BP ratios were around 0.6 and less than 0.1, respectively (9). Nucleoside reverse transcriptase inhibitors accumulate to various extents in the male genital tract, with SP-to-BP ratios of 2.28 and 6.67 for lamivudine and zidovudine, respectively (9, 10). Available data on tenofovir (TFV) SP concentrations are sparse, although this drug is recommended as a preferred choice in first-line regimens for HIV treatment and postexposure prophylaxis and a large body of evidence from trials using TFV-based regimens now supports the concepts of “treatment as prevention” and “preexposure prophylaxis” (11, 12). Four studies have suggested an accumulation of TFV in the male genital tract to different extents (13–16). Mean TFV SP-to-BP concentrations ratios have been estimated at 2.8 ( $n = 4$  men) and 3.3 ( $n = 15$ men) for various sampling times (13, 14). Mean TFV SP-to-BP concentration ratios at 24 h ( $C_{24}$ ) were estimated at 4.4 for a single dose and 5.1 at steady state ( $n = 9$ men) (15). Thus, the TFV SP-to-BP concentration ratio appears to be variable depending on the time elapsed between drug intake and sampling. Another study has focused on a TFV single-dose administration and has reported an SP-to-BP exposures ratio of 1.0 (16). However, that study included a small number of subjects ( $n = 8$ men) and examined only the final phase of decay of TFV pharmacokinetics (PK) (from 24 h to 14 days). Furthermore, the blood-testis barrier, composed mainly of Sertoli cells, is responsible for the protection of developing germ cells from exposure to xenobiotics. Several efflux transporters for which TFV is potentially a substrate, such as P-gp and MRP4, have been shown to be present and active at the blood-testis barrier (17). Therefore, any genetic polymorphism of one of those carriers could modify SP concentrations and explain a part of the variability observed in TFV transfer into the male genital tract and thus possibly HIV shedding in this compartment.

No study has reported the penetration of TFV in the male genital tract as the SP-to-BP exposure ratio at steady state under the conditions of chronic once-daily administration. The aims of our work were (i) to describe TFV BP and SP pharmacokinetics by a population approach, (ii) to evaluate the TFV distribution in the male genital tract by use of an SP-to-BP exposure ratio at steady state, (iii) to assess the role of genetic polymorphism in the variability of TFV transfer into the male genital tract, and (iv) to assess the effect of TFV exposure levels in the male genital tract on spVL.

## RESULTS

**Demographic data.** Data from 129 and 123 men were available for BP and SP analyses, respectively. Table 1 summarizes the patients' demographic and biological

**TABLE 2** TFV population pharmacokinetic parameters for plasma and seminal plasma

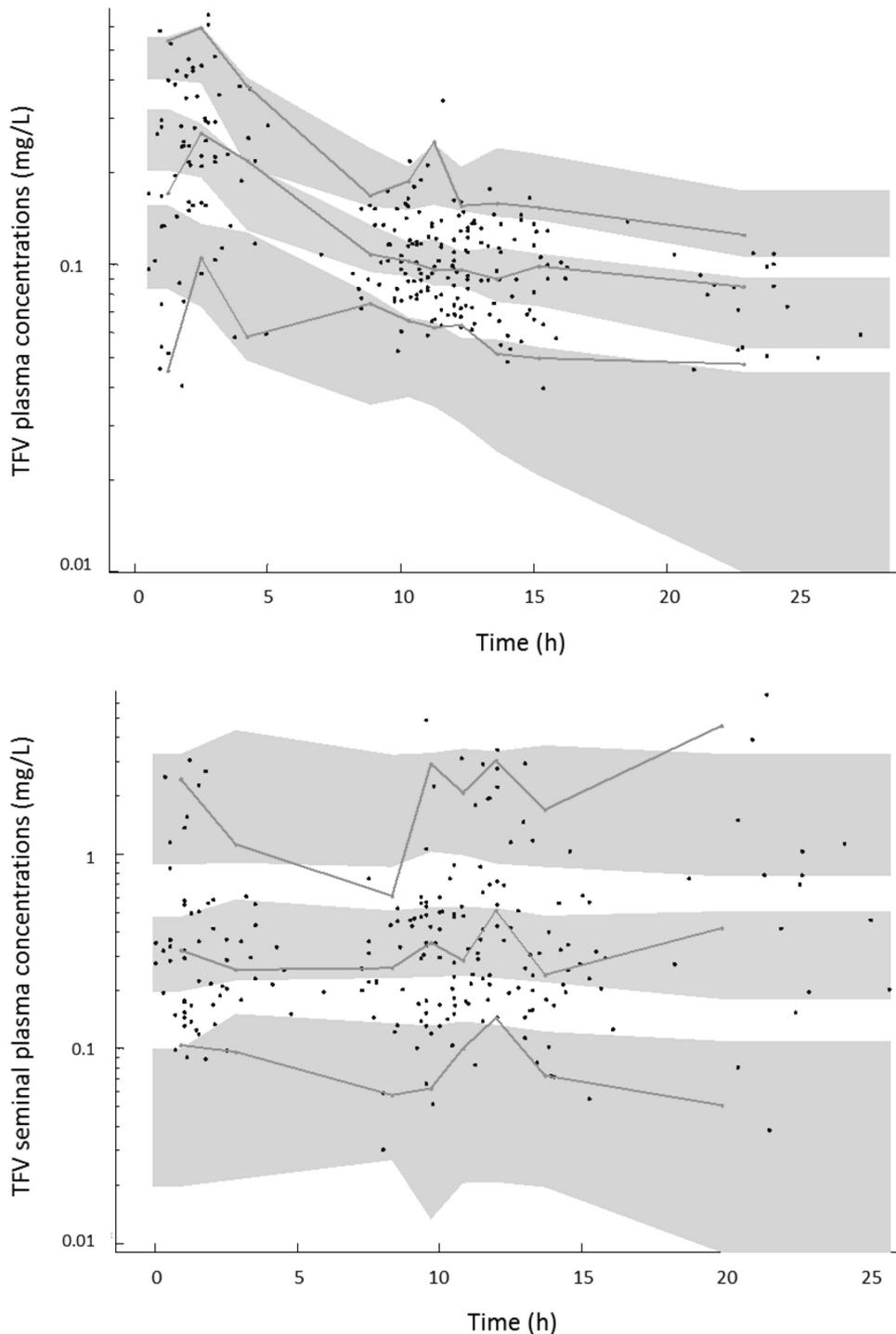
Parameter <sup>a</sup>	Estimate	RSE (%)
Structural model		
$k_a$ ( $\text{h}^{-1}$ )	1.35	41
CL/F (liters $\cdot$ $\text{h}^{-1}$ )	45.8	3
$V_c/F$ (liters)	268	16
Q/F (liters $\cdot$ $\text{h}^{-1}$ )	197	39
$V_p/F$ (liters)	1630	43
$\beta_{\text{LPV/r}}$ on CL/F	0.591	13
$\beta_{\text{CLCR}}$ on CL/F	0.269	34
$k_{1e}$ ( $\text{h}^{-1}$ )	0.0963	28
$k_{e1}$ ( $\text{h}^{-1}$ )	0.0339	29
Statistical model		
$\omega$ CL/F	0.146	48
$\omega$ Q/F	1.94	45
$\omega$ $k_{e1}$	0.889	7
$\sigma_{\text{plasma}}$	0.263	8
$\sigma_{\text{seminal plasma}}$	0.374	7

<sup>a</sup>Abbreviations:  $k_a$ , absorption rate constant; CL/F, apparent elimination clearance;  $V_c/F$ , apparent central volume of distribution; Q/F, apparent intercompartmental clearance;  $V_p/F$ , apparent peripheral volume of distribution;  $F$ , unknown bioavailability;  $\beta_{\text{LPV/r}}$  on CL/F, influential factor of lopinavir/ritonavir coadministration on CL/F;  $\beta_{\text{CLCR}}$  on CL/F, influential factor of creatinine clearance on CL/F;  $k_{1e}$ , plasma to seminal plasma transfer rate constant;  $k_{e1}$ , seminal plasma elimination rate constant;  $\omega$ , interindividual variability estimates;  $\sigma_{\text{plasma}}$ , proportional residual variability estimate for plasma concentrations;  $\sigma_{\text{seminal plasma}}$ , proportional residual variability estimate for seminal plasma concentrations.

characteristics. Tenofovir disoproxil fumarate (TDF) was associated with emtricitabine (FTC) for 94.5% of men and with abacavir for 4.7% of men. TDF was combined with either one nonnucleoside reverse transcriptase inhibitor, one ritonavir-boosted protease inhibitor, or raltegravir in 49%, 31%, and 14% of these men, respectively. The most frequent combination was TDF plus FTC plus efavirenz (55 men out of 129). Ninety-five percent of patients respected the 48-hour abstinence period before semen sampling, and 13% had clinical symptoms suggestive of potential sexually transmitted infections.

**Population pharmacokinetics.** A total of 248 TFV BP and 217 TFV SP concentrations at steady state were available for the analysis. A two-compartment model with first-order absorption and elimination with an effect compartment linked to the central compartment satisfactorily described the TFV plasma and seminal plasma concentrations. For seminal plasma, the best fits and objective function value (OFV) were obtained by estimating different input and output rate constants for the effect compartment. Adding a transit compartment between the central and effect compartments did not improve the fit. The pharmacokinetic parameters of this model were the absorption rate constant ( $k_a$ ), apparent elimination clearance (CL/F), central volume of distribution ( $V_c/F$ ), intercompartmental clearance (Q/F), peripheral volume of distribution ( $V_p/F$ ), BP-to-SP transfer rate constant ( $k_{1e}$ ), and SP elimination rate constant ( $k_{e1}$ ), with  $F$  being the unknown bioavailability.

The residual variability was described by a proportional error model for both BP and SP. Interindividual variabilities were retained for CL/F, Q/F, and  $k_{e1}$ . For BP pharmacokinetics, the most significant decrease in OFV was obtained by adding the effect of lopinavir/ritonavir (LPV/r) on CL/F. After the inclusion of LPV/r on CL/F, the addition of creatinine clearance (CL<sub>CR</sub>) on CL/F significantly decreased the OFV. The effect of darunavir/ritonavir on CL/F was significant for the upward phase but not for the backward phase. Thus, the final covariate model for BP modeling was  $\text{CL}/F = \theta_{\text{CL}/F} \times \beta_{\text{LPV/r}} \times (\text{CL}_{\text{CR}}/113)^{\beta_{\text{CLCR}}}$ , with  $\theta_{\text{CL}/F}$  the typical value of CL/F for a patient with a median CL<sub>CR</sub> value of 113 ml  $\cdot$  min<sup>-1</sup> and no coadministration of LPV/r. For TFV SP modeling, the effects of covariates on  $k_{e1}$  did not significantly decrease the OFV. Table 2 summarizes the final population pharmacokinetic estimates for the BP and SP model, including the relative standard errors.



**FIG 1** Validation of the final model by the visual predictive check (VPC), showing comparison between the 5th, 50th, and 95th percentiles of observed data (lines), the 90% confidence interval of simulated percentiles (areas), and the observed data (circles) for TFV plasma concentrations (top panel) and TFV seminal plasma concentrations (bottom panel) on a log scale.

**Model evaluation and validation.** Table 2 summarizes the final model parameters. All the parameters were well estimated given the low relative standard error (RSE) values. The visual predictive check (VPC) of the final model shows that the 5th, 50th, and 95th percentiles of observed data are well included in the 90% confidence interval (CI) of the 5th, 50th, and 95th simulated percentiles both for BP and SP (Fig. 1).

**TABLE 3** TFV plasma and seminal plasma exposures

Parameter	Plasma AUC <sub>0–24</sub>	Seminal plasma AUC <sub>0–24</sub>	SP-to-plasma AUC <sub>0–24</sub> ratio
Sample size	248	217	217
Median (mg · liter <sup>-1</sup> · h)	2.97	7.01	2.24
Range (mg · liter <sup>-1</sup> · h)	2.08–7.06	1.25–107.29	0.53–34.13

**TFV blood plasma and seminal plasma exposures.** Both the TFV BP area under the concentration-time curve from 0 to 24 h (AUC<sub>0–24</sub>) and C<sub>24</sub> were higher for patients receiving LPV/r than for patients without LPV/r coadministration (Mann-Whitney-Wilcoxon test,  $P < 10^{-4}$ ). The median BP AUC<sub>0–24</sub> was 5.10 mg · liter<sup>-1</sup> · h and 2.95 mg · liter<sup>-1</sup> · h for patients receiving LPV/r or not, respectively. Seminal AUC<sub>0–24</sub> and C<sub>24</sub> were close between patients receiving LPV/r or not (Mann-Whitney-Wilcoxon test,  $P = 0.61$  and  $0.67$ , respectively). The median SP AUC<sub>0–24</sub> was 6.95 mg · liter<sup>-1</sup> · h for patients without LPV/r and 7.70 mg · liter<sup>-1</sup> · h for patients receiving LPV/r.

TFV concentrations in the male genital tract were higher than BP concentrations. The median C<sub>24</sub> (range) was 0.26 (0.03 to 4.42) mg · liter<sup>-1</sup> for SP and 0.074 (0.030 to 0.190) mg · liter<sup>-1</sup> for BP. TFV BP and SP AUC<sub>0–24</sub> and SP-to-BP exposures ratios are summarized in Table 3. The median (range) TFV BP AUC<sub>0–24</sub> was 2.97 (2.08 to 7.06) mg · liter<sup>-1</sup> · h, the median (range) TFV SP AUC<sub>0–24</sub> was 7.01 (1.25 to 107.3) mg · liter<sup>-1</sup> · h, and the median (range) SP-to-BP AUC<sub>0–24</sub> ratio was 2.24 (0.53 to 34.1). TFV penetration in the male genital tract was highly variable, with a SP-to-BP AUC<sub>0–24</sub> ratio coefficient of variation (CV) of 125%. A total of 28 SP-to-BP AUC<sub>0–24</sub> ratios out of 217 were lower than 1 (12.9% of ratios). TFVSP AUC<sub>0–24</sub> values were higher than BP AUC<sub>0–24</sub> values for 87.1% of the men.

**Association between genetic polymorphism and TFV transfer into the male genital tract.** Among the 129 patients who gave consent for genetic analysis and for whom germ line DNA was available, 122 were included in the genetic analysis (1 patient had genotyping of insufficient quality, with 60% of the single nucleotide polymorphisms [SNPs] genotyped, and 6 patients had a DNA quantity not suitable for a large-scale genotyping). From the 121 SNPs initially selected, 3 SNPs were filtered out after quality control due to a call rate of <90% (1 SNP, i.e., rs17300696 in the ABCC4 gene) or to a minor allele frequency of <10% (2 SNPs, i.e., rs45610534 and rs45573936 in the ABCC4 and SLC29A1 genes, respectively). Finally, 118 SNPs were included in the genetic analysis (see Table S1 in the supplemental material). The  $\eta$ -shrinkage of the TFV transfer constant rate ( $k_{e1}$ ) was 0.067, indicating that the empirical Bayesian estimates were reliable. In order to use a normally distributed outcome, the square root value of the  $k_{e1}$  parameter was used to test the association with genetic polymorphism (Shapiro-Wilk normality test,  $P = 0.14$ ). From the 118 SNPs tested, 85 effective independent tests were actually estimated; the significance threshold was then fixed to 0.0006. After correction for multiple testing, none of the SNPs were significantly associated with the TFV transfer rate constant (see Fig. S1 in the supplemental material). Interestingly, the most significant SNP was rs10508017 in the ABCC4 gene, coding for a transport protein in the blood-testis barrier in a recessive genetic model ( $P$  value = 0.001).

**Impact of TFV exposures on seminal plasma HIV load.** A seminal plasma HIV load was detectable (>100 copies · ml<sup>-1</sup>) for 15 patients out of 123 (12.2%) and for 17 samples out of 215 (7.9%). The median (range) for the detectable HIV load in seminal plasma was 242 (118 to 1475) copies · ml<sup>-1</sup>. Median SP AUC<sub>0–24</sub> and SP-to-BP AUC<sub>0–24</sub> ratios were lower for patients with detectable spVL than for patients with undetectable spVL (4.78 versus 7.02 mg · liter<sup>-1</sup> · h for SP AUC<sub>0–24</sub>; 1.58 versus 2.28 for SP-to-BP exposure ratio) but the difference did not reach significance ( $P = 0.32$  and  $P = 0.25$ , respectively, by the Wilcoxon signed-rank test). No association between TFV distribution in the male genital tract and spVL was shown by the receiver operating characteristic (ROC) curve analyses. The areas under the ROC curves (95% CI) were very low:

**TABLE 4** Mixed-effects logistic regressions for spVL detectability

Parameter <sup>a</sup>	P value
TFV SP AUC <sub>0-24</sub>	0.808
TFV SP AUC <sub>0-24</sub> + STI symptoms	0.809
TFV SP AUC <sub>0-24</sub> + Herpes infection symptoms	0.965
TFV SP-to-plasma AUC <sub>0-24</sub> ratio	0.816
TFV SP-to-plasma AUC <sub>0-24</sub> ratio + STI symptoms	0.180
TFV SP-to-plasma AUC <sub>0-24</sub> ratio + Herpes infection symptoms	0.768
TFV SP-to-plasma AUC <sub>0-24</sub> ratio	0.768
TFV SP-to-plasma AUC <sub>0-24</sub> ratio + STI symptoms	0.965
TFV SP-to-plasma AUC <sub>0-24</sub> ratio + Herpes infection symptoms	0.775
	0.179

<sup>a</sup>Abbreviations: SP, seminal plasma; STI, sexually transmitted infections.

0.57 (0.43 to 0.71) for seminal plasma AUC<sub>0-24</sub> values and 0.58 (0.44 to 0.73) for SP-to-BP AUC<sub>0-24</sub> ratios. Using mixed-effects logistic regressions with random effect on individual, the impact of TFV distribution in the male genital tract, evaluated by SP AUC<sub>0-24</sub> and SP-to-BP AUC<sub>0-24</sub> ratio, on spVL was not significant ( $P = 0.81$  and  $0.77$ , respectively). The influence of TFV distribution in the male genital tract remained not significant after adjusting the multivariate logistic regression on confounding factors (clinical symptoms of potential sexually transmitted infections and herpes infection) (Table 4). The effect of combined TFV-FTC seminal plasma AUC<sub>0-24</sub> or SP-to-plasma AUC<sub>0-24</sub> ratios on spVL was not significant ( $P$  value for interaction =  $0.98$  for SP AUC<sub>0-24</sub>;  $P$  value for interaction =  $0.73$  for SP-to-BP AUC<sub>0-24</sub> ratios).

## DISCUSSION

This is the first TFV BP and SP population pharmacokinetic study. Concentrations were satisfactorily described by a two-compartment model connected to an effect compartment with different input and output constants. The effects of CL<sub>CR</sub> and LPV/r coadministration on TFV BP pharmacokinetics were significant, as previously published (18–20). The final model was evaluated by visual predictive check, and the BP AUC<sub>0-24</sub> was close to previous published values in adults (mean AUC<sub>0-24</sub> =  $2.95 \text{ mg} \cdot \text{liter}^{-1} \cdot \text{h}$  versus  $3.0 \text{ mg} \cdot \text{liter}^{-1} \cdot \text{h}$  [18] or  $2.87 \text{ mg} \cdot \text{liter}^{-1} \cdot \text{h}$  [19]). Despite the sparse nature of the PK data, no significant shrinkage was pointed out, especially regarding the seminal plasma elimination rate constant, indicating that individual parameters were reasonably identified. TFV SP concentrations were higher than TFV BP concentrations, with a median SP C<sub>24</sub> of  $0.26 \cdot \text{liter}^{-1}$  compared to a median C<sub>24</sub> of  $0.074 \text{ mg} \cdot \text{liter}^{-1}$  in BP. One TFV single-dose study has reported median TFV SP and BP C<sub>24</sub> values of  $0.023 \text{ mg} \cdot \text{liter}^{-1}$  and  $0.041 \text{ mg} \cdot \text{liter}^{-1}$ , respectively (16). The discrepancy between the median C<sub>24</sub> values probably results from the steady-state condition and an accumulation of TFV in the male genital tract with repeated administrations. Other studies have reported higher TFV concentrations in SP, with SP-to-BP concentration ratios of 2.8 and 3.3 (13, 14). The mean C<sub>24</sub> ratio at steady state was reported to be 5.1 (15), close to the mean C<sub>24</sub> ratio at steady state of 6.6 estimated in our study. Because these concentrations ratios are highly variable depending on the time elapsed between drug intake and sampling, the SP-to-BP AUC ratio would be more representative of the penetration in the male genital tract. The median SP-to-BP exposures ratio was 2.24 (25th to 75th percentile, 1.47 to 4.17). Patterson et al. have reported an SP-to-BP exposure ratio of 1.0 (25th to 75th percentile, 0.6 to 1.4). However, they reported AUC from 24 h to 14 days for a single-dose administration and focused only on the final decay phase after 24 h (16).

Regarding other tissues, the TFV distributions in the cerebrospinal fluid and female genital tract have been reported. Low TFV cerebrospinal fluid concentrations have been reported, with concentrations being 5% of the BP concentrations (21). The TFV distribution in the female genital tract has been evaluated by noncompartmental analyses, and TFV cervicovaginal fluid-to-BP AUC ratios were estimated at 0.75 and 2.6, respectively (16, 22). Another study using a modeling approach has reported a cervicovaginal

fluid-to-BP AUC ratio of 1.6 (23). Thus, the distribution of TFV appears to be variable and specific for each tissue.

The TFV distribution in the male genital tract was variable, with a substantial interindividual variability (0.89) for the SP elimination rate constant ( $k_{e1}$ ). No effect of any available covariate (including genetic polymorphism) could explain the interindividual variability on  $k_{e1}$ . The TFV SP-to-BP  $AUC_{0-24}$  ratio was highly variable, with a coefficient of variation of 125%. Accordingly, 12.9% of SP-to-BP  $AUC_{0-24}$  ratios were lower than 1, showing that TFV seminal plasma  $AUC_{0-24}$  values were not systematically higher than BP  $AUC_{0-24}$  values.

TFV is eliminated mainly by the kidney, via a combination of glomerular filtration and active tubular secretion involving transporter proteins. TFV has been described to be a substrate of organic anion transporter 1 (OAT1), and multidrug resistance-associated proteins 4 (MRP4) (24) and 7 (MRP7) (25). A genetic polymorphism in the ABCC4 gene (MRP4) has been associated with higher intracellular concentrations of TFV-diphosphate (26). Another study has shown that genetic variability in the ABCC10 gene (MRP7) may influence TFV renal tubular transport and contribute to the development of tubular dysfunction (25). Thus, genetic polymorphisms in the renal transporters of TFV could influence TFV pharmacokinetics. Robillard et al. have studied the expression of membrane transporters in human Sertoli cells, one of the main constituents of the blood-testis barrier. The expression and activity of MRP4 have been confirmed (17). Interestingly, in our study, the most significant SNP associated with TFV transfer to the male genital tract was rs10508017 in the ABCC4 gene, coding for the MRP4 transport protein. However, this SNP did not cross the prespecified significance level ( $P = 0.001$ ). Further studies will be needed to confirm this hypothesis.

In the Evarist study, BP HIV RNA was undetectable for all enrolled men, while SP HIV RNA was detectable in 7.9% of samples. This discordance between BP HIV RNA level and SP HIV RNA was close to the mean percentage previously reported (10%) (7). TFV penetration in the male genital tract was variable, and TFV SP  $AUC_{0-24}$  values were lower than TFV BP  $AUC_{0-24}$  values in 12.9% of the men. However, the low SP  $AUC_{0-24}$  ratios were not associated with SP HIV RNA positivity. In a previous study, FTC SP  $AUC_{0-24}$  values were higher than the efficacy target FTC BP  $AUC_{0-24}$  in more than 99% of men and were not reported as the main factor influencing spVL positivity (27). The TFV distribution in the male genital tract was more variable than the FTC distribution (CVs of SP-to-BP  $AUC_{0-24}$  ratios of 125 and 54.7%, respectively), with ratios lower than 1 more prevalent (12.9% versus <1%). The influence of the TFV SP  $AUC_{0-24}$  or SP-to-BP  $AUC_{0-24}$  ratio on spVL was not significant. This remained not significant when symptoms of sexually transmitted diseases, previously associated with seminal plasma HIV shedding in men treated by cART, were taken into account (5). Similarly, the impact of TFV-FTC exposures on spVL was not significant. The nonsignificant results regarding exposure and response may be due to the fact that only 8% patients had detectable seminal viral load. A lack of power cannot be ruled out in our study. Aside from the lack of power, the absence of association between spVL and TFV penetration in the male genital tract could be due to the assessment of only a part of the overall drug combination. Therefore, these results suggest a substantial effect of other concomitant ARV drug exposures in seminal plasma. Viral particle transfer coming from another compartment could also explain these results. Actually, it was not shown in our study that the measured spVLs were produced locally; therefore, these viruses can also reflect a residual replication somewhere in the body, particularly in another compartment with perhaps insufficient drug levels. In the same way, Ghosn et al. have reported that the size of the blood HIV-1 reservoir was highly associated with spVL detection (28).

In conclusion, this work presents the first population model describing TFV pharmacokinetics in blood plasma and seminal plasma in a large population of HIV-1-infected men. TFV seminal plasma concentrations were higher than blood plasma concentrations. TFV accumulated in the seminal plasma in a majority of men (87.8%).

The median SP-to-BP  $AUC_{0-24}$  ratio was 2.24. Despite TFV SP exposures higher than BP exposures in a majority of men, an spVL was detectable for 12.2% of patients.

## MATERIALS AND METHODS

**Study.** The Evarist-ANRS EP 49 study's main objective was to evaluate the proportion of men having an undetectable blood HIV RNA BP VL ( $<50$  copies  $\cdot$  ml $^{-1}$ ) and a concomitant detectable viral load in semen (28). Men older than 18 years having sex with men, infected by HIV-1, receiving the same antiretroviral treatment for at least 3 months, and having an undetectable BP viral load ( $<50$  copies  $\cdot$  ml $^{-1}$ ) for at least 6 months were eligible. The Evarist-ANRS EP 49 study protocol was approved by the Ethics Committee of Bicêtre Hospital. All the patients have signed written informed consent forms.

**Patients, treatment, and sampling.** The present study population included HIV-1-infected men from the Evarist-ANRS EP 49 study receiving tenofovir disoproxil fumarate (TDF) as part of their cART. Patients received a 300-mg dose of TDF once daily. Two paired blood and semen samples were planned to be collected at two different visits (at day 0 and day 30). The time between drug intake and sampling times was variable among patients. The median (interquartile range) for the delay between dose intake and blood sampling was 11.1 h (3.7 h to 13.4 h) and 10.7 h (4.0 h to 12.5 h), respectively, for day 0 and day 30. The delay between dose intake and semen sampling was 10.5 h (3.5 h to 13 h) and 10 h (4.2 h to 12.1 h), respectively, for day 0 and day 30. The semen sampling was done 35 min (15 to 74.8 min) before the blood sampling. The seminal plasma HIV RNA load (lower limit of quantification [LLOQ], 100 copies  $\cdot$  ml $^{-1}$ ) was measured during these visits. For each man, demographic (body weight and age) and biological (serum creatinine) characteristics were recorded. Creatinine clearance ( $CL_{CR}$ ) was estimated using the Cockcroft-Gault formula. Associated antiretroviral drugs, respect of a 48-hour abstinence period before semen sampling, and clinical symptoms of potential sexually transmitted infections were also recorded.

**Analytical method.** Tenofovir BP and SP concentrations were measured using a validated high-performance liquid chromatography-tandem mass spectrometry method (29). The LLOQ was 5 ng  $\cdot$  ml $^{-1}$  and 3.13 ng  $\cdot$  ml $^{-1}$  in BP and SP, respectively. A calibration curve was constructed in either spiked blank BP or SP over concentration ranges of 5 to 1,000 ng  $\cdot$  ml $^{-1}$  (BP) and 3.13 to 1,000 ng  $\cdot$  ml $^{-1}$  (SP). Serum creatinine concentrations were measured using the colorimetric method of Jaffe on a Cobas 8000 (Roche/Hitachi).

**Genetic analysis.** Written consent for genetic analysis has been obtained from 123 patients for whom the plasma TDF concentration has been measured. DNAs were extracted from blood using a Qiagen kit following the manufacturer's instructions (Qiagen GmbH, Germany). Genes coding for transport proteins for which TFV is known to be a substrate were primarily selected (30). Among these genes, only those that were expressed in the blood-testis barrier were finally selected for further genetic analysis (<http://www.proteinatlas.org/>). The selected genes (proteins) were as follows: SLC28A2 (CNT-2), SLC29A1 (OAT-1), SLC29A2 (OAT-2), SLC22A1 (OCT-1), SLC22A2 (OCT-2), ABCC2 (MRP-2), ABCC4 (MRP-4), and ABCC10 (MRP-7).

We selected tag SNPs (tSNPs) capturing the common genetic variations of these 8 genes based on variation catalogued in dbSNP from the European population, using the LD TAG SNP selection tool of the SNPinfo web server (31) with the following parameters: (i) a minor allele frequency (MAF) of  $>20\%$  and (ii) a linkage disequilibrium threshold of  $r^2 > 0.8$ .

In addition to these tSNPs, a few SNPs were also included based on literature review. Both rs3742106 and rs1751034 in ABCC4 have been correlated with higher plasma tenofovir concentrations (32, 33), as well as rs11854484 in SLC28A2 (CNT-2) (34). Furthermore, rs9349256 and rs2125739 in ABCC10 were associated with kidney tubular dysfunction in patients treated with TDF (25). Finally, a total of 121 SNPs composed of tSNPs and SNPs from the literature were genotyped (Integrage, Evry, France) using Fluidigm technology.

**Population pharmacokinetic modeling strategy.** Data were analyzed by a population approach, using the nonlinear mixed-effect modeling software program Monolix version 4.1.4 (available at [www.lixoft.eu](http://www.lixoft.eu)). Parameters were estimated using the stochastic approximation expectation maximization (SAEM) algorithm combined with a Markov chain Monte Carlo (MCMC) procedure (35). The maximum number of iterations for each stage (K1 and K2) was fixed at 500 and 200, respectively. The number of Markov chains was fixed at 5 for all estimations.

In a first step, the TFV BP concentrations were modeled and pharmacokinetic (PK) parameters were estimated. Then, these parameters were fixed in order to investigate TFV SP PK modeling. In a final step, all the BP and SP parameters were estimated.

One- and two-compartment models with linear absorption and elimination were investigated as structural models to describe TFV BP pharmacokinetics. The addition of both an additional compartment linked to the central compartment by a first-order process and an effect compartment was tested to describe TFV SP pharmacokinetics. The effect compartment was modeled as a virtual compartment of negligible volume, not modifying the compartmental model in BP. Several models were explored: (i) the effect compartment was connected to the central compartment by a first-order process with the same or different input and output constants, (ii) a transit compartment was inserted between the central and effect compartments, and (iii) the effect compartment was connected to the BP peripheral compartment.

Several error models (proportional, additive, and mixed) were tested to describe the residual variabilities ( $\varepsilon$ ). Interindividual variabilities (IIV or  $\eta$ ) were assumed to be exponential. The effect of patient continuous covariates on pharmacokinetic parameters was tested according to a power model centered on the median. The effect of categorical covariates was tested according to the equation  $CL/F = \theta_{CL/F} \times \beta CO^{\alpha}$ , where  $\theta_{CL/F}$  is the typical value of apparent elimination clearance for a subject without the

covariate,  $CO = 0$  for the reference value of  $CL/F$ ,  $CO = 1$  for the value of  $CL/F$  with the covariate, and  $\beta_{CO}$  is the estimated influential factor of the binary covariate. The effect of combined antiretroviral drugs was tested individually, e.g., the effect of lopinavir alone, or by pharmaceutical class, e.g., the effect of protease inhibitor class. The influence of covariates was evaluated via an upward-backward model building procedure.

The objective function value (OFV) was used to test different hypotheses regarding the structural model, the structure of the variance-covariance matrix for IIV, the residual variability models, and the covariate effect(s) on pharmacokinetic parameter(s). A covariate was finally retained in the model if its effect was biologically plausible, if it produced a reduction in the variability of the pharmacokinetic parameter (IIV), and if the OFV was decreased by at least 3.84 ( $\chi^2$  with 1 df,  $P < 0.05$ ) in the upward phase and was increased by more than 6.63 in the backward phase ( $\chi^2$  with 1 df,  $P < 0.01$ ).

**Model evaluation.** Goodness of fit was evaluated using the following graphs: observed concentrations versus population and individual predictions, weighted residuals versus time, and weighted residuals versus population predictions.

For validation, a visual predictive check (VPC) was performed (36). The model was used to simulate 500 vectors of parameters for each sampling time. Simulated TFV concentrations and observed data were compared: the 5th, 50th, and 95th percentiles of observed data were overlaid on the 90% confidence interval of the 5th, 50th, and 95th simulated percentiles, and a visual inspection was performed.

**Association between genetic polymorphism and TFV transfer into the male genital tract.** SNPs that passed the quality control checks, i.e., (i) Hardy-Weinberg equilibrium with  $P > 0.05$ , (ii) frequency test (MAF  $> 0.1$ ), and (iii) call rate  $>90\%$ , were further tested for association with the TFV transfer rate constant (derived from the estimated individual pharmacokinetic parameters). Using a linear regression model, all possible genetic models were tested (additive, dominant, and recessive). Accounting for correlation due to linkage disequilibrium between the different SNPs tested, the number of independent tests was then estimated using the Genetic Type I Error Calculator software (37) in order to derive the significance threshold for multiple testing issues.

**TFV BP and SP exposures.** TFV blood plasma (BP) and seminal plasma (SP) exposures ( $AUC_{0-24}$ ) were derived from the estimated individual pharmacokinetic parameters. Distribution in the male genital tract was assessed by calculating SP-to-BP  $AUC_{0-24}$  ratios. The percentage of exposure ratios lower than 1 was calculated.

**Impact of TFV exposures on SP HIV RNA load.** To assess the association between TFV SP  $AUC_{0-24}$  or TFV SP-to-BP  $AUC_{0-24}$  ratio and spVL, ROC curves were determined using R software (38). The area under the ROC curve value associated with its 95% confidence interval was estimated. Mixed-effects logistic regressions with random effect on individuals were performed in order to evaluate the impact of TFV SP  $AUC_{0-24}$ , TFV SP-to-BP  $AUC_{0-24}$  ratio, and potentially significant cutoffs obtained from the ROC curves on spVL (39). Using the results of a previous study on emtricitabine (FTC) penetration in the male genital tract (27), the impact of TFV-FTC seminal plasma  $AUC_{0-24}$  or SP-to-BP  $AUC_{0-24}$  ratios on spVL was also investigated.

## SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AAC.02062-16>.

**TEXT S1**, PDF file, 0.1 MB.

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