



Race/Ethnicity and Protease Inhibitor Use Influence Plasma Tenofovir Exposure in Adults Living with HIV-1 in AIDS Clinical Trials Group Study A5202

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ABSTRACT AIDS Clinical Trial Group study A5202 (ClinicalTrials.gov identifier NCT00118898) was a phase 3b, randomized, partially blinded equivalence study of open-label atazanavir/ritonavir or efavirenz, plus either placebo-controlled tenofovir disoproxil fumarate/emtricitabine or abacavir/lamivudine, in treatment-naïve adults living with HIV-1, evaluating efficacy, safety, and tolerability. We report an analysis of the contribution of participant characteristics to the disposition of tenofovir plasma concentrations. Tenofovir concentration data from a total of 817 individuals (88% of the total number of eligible patients randomly assigned to receive treatment in the TDF-containing arms of A5202) were available for analysis. Pharmacokinetic analysis was performed using nonlinear mixed-effects modeling. One- and two-compartment models with first-order absorption and first-order elimination were evaluated. An exponential error model was used for examination of interindividual variability (IIV), and a proportional and mixed-error model was assessed for residual variability. The final structural model contained two compartments with first-order absorption and elimination. IIV was estimated for apparent clearance (CL/F) and the first-order absorption rate constant (k_a), and a proportional residual variability model was selected. The final mean parameter estimates were as follows: $k_a = 2.87 \text{ h}^{-1}$, CL/F = 37.2 liters/h, apparent volumes of the central and peripheral compartments = 127 and 646 liters, respectively, and apparent intercompartmental clearance = 107 liters/h. In addition to race/ethnicity, creatinine clearance and assignment to atazanavir/ritonavir or efavirenz were significantly associated with CL/F ($P < 0.001$). In conclusion, race/ethnicity is associated with tenofovir oral CL in HIV-1 positive, treatment-naïve adults. This covariate relationship raises questions about the possibility of differences in efficacy and risk of adverse events in different patient populations and suggests that examining preexposure prophylaxis regimens and tenofovir exposure in different race/ethnicity groups be considered.

KEYWORDS pharmacokinetics, population pharmacokinetics, tenofovir

Citation Bednasz CJ, Venuto CS, Ma Q, Daar ES, Sax PE, Fischl MA, Collier AC, Smith KY, Tierney C, Acosta EP, Mager DE, Morse GD, on behalf of the AIDS Clinical Trials Group Study A5202 Team. 2019. Race/ethnicity and protease inhibitor use influence plasma tenofovir exposure in adults living with HIV-1 in AIDS Clinical Trials Group study A5202. *Antimicrobial Agents Chemother* 63:e01638-18. <https://doi.org/10.1128/AAC.01638-18>.

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Received 3 August 2018

Returned for modification 10 September 2018

Accepted 11 December 2018

Accepted manuscript posted online 14 January 2019

Published 27 March 2019

Tenofovir disoproxil fumarate (TDF) is an adenosine-analog nucleotide reverse transcriptase inhibitor (NRTI) prodrug that exhibits activity against HIV-1 and hepatitis B virus infections. TDF is converted to its active intracellular form, tenofovir diphosphate, in a stepwise manner: (i) TDF is converted to tenofovir (TFV) in the intestinal lumen and plasma by diester hydrolysis, and (ii) TFV is internalized intracellularly and subsequently phosphorylated into tenofovir monophosphate and then to its active metabolite, tenofovir diphosphate (1, 2). Tenofovir has been examined in those living with HIV and healthy volunteers and has demonstrated that renal function and concomitant use with ritonavir-boosted HIV-1 protease inhibitors affect plasma TFV concentrations (3–6).

The AIDS Clinical Trials Group study A5202 (ClinicalTrials.gov identifier NCT00118898) randomly assigned 1,857 treatment-naïve HIV-1-infected adults to one of the following once-daily regimens: open-label atazanavir and ritonavir (ATV/r; 300/100 mg) or efavirenz (EFV; 600 mg), plus either placebo-controlled tenofovir disoproxil fumarate/emtricitabine (TDF/FTC; 300/200 mg) or abacavir/lamivudine (ABC/3TC; 600/300 mg). Plasma concentration data for each of the antiretrovirals were collected from the majority of study participants for further investigation of differences in the pharmacokinetics of subpopulations. Included in the prespecified pharmacology-related secondary objectives was the exploration of the association of ethnicity and other host factors with the disposition of antiretroviral (ARV) agents. It is critical to determine whether clinically relevant differences in ARV drug exposure exist in potential patient subpopulations, since variability in drug exposure may be associated with differences in ARV drug toxicity and virologic response. In this report, we investigated TFV pharmacokinetics using sparse sampling and population pharmacokinetic modeling to conduct a covariate analysis in exploration of significant host factors that may play a role in the time course of TFV exposure.

RESULTS

Pharmacokinetic data from a total of 817 individuals (88%) were available for analysis. The majority of participants had 3 samples drawn (range, 1 to 5), with a total of 2,166 evaluable plasma concentrations within approximately 6 months of beginning antiretroviral treatment. Information on the previous 3 days of TDF dosing prior to pharmacokinetic sampling was available for 98% of the 2,166 evaluable plasma concentrations, in which 42 and 18 sample collections reported a 2-day difference between the dose prior to sampling and the second dose prior to sampling and between the second and third dose prior to sampling, respectively. One participant reported a 3-day window between the dose prior to sampling and the second dose prior to sampling.

Population pharmacokinetic model. A two-compartment model with first-order absorption and elimination was ultimately the most appropriate choice to describe the data. A mixture model identified a subpopulation with large residual variability ($n = 30$), and a total of 78 observations were removed from the data set for model building. The demographics of the remaining participants ($n = 787$) used in the pharmacokinetic analysis are given in Table 1. The final model included interindividual variability (IIV) terms on apparent clearance (CL/F) and the first-order absorption rate constant (k_a), and a proportional model was selected for residual variability. The base model was able to accommodate IIV on k_a in addition to CL/F, but the only covariate that was biologically plausible to affect the absorption rate constant (i.e., age) was not significant. Whereas IIV on k_a and CL/F during forward selection would not allow estimation of race/ethnicity as a covariate on CL/F with 1 or 2 degrees of freedom, forward selection was continued with IIV on CL/F only, and IIV on k_a was added during the multivariable evaluation. This allowed for greater precision of parameter estimates, clinically valuable covariate information to be included in the model, and retention of IIV on k_a . In the final model, the IIV variability on CL/F and k_a were 18% and 85% (% coefficient of variation [% CV]), with information on covariate model development summarized in Table 2. The eta distribution associated with the absorption rate constant had moderate asymmetrical shrinkage (57%) due to missing data in the absorption phase.

TABLE 1 Demographics of study participants

Demographic	Value (n = 787) ^a
Treatment arm (3rd drug), no. (%) of participants	
ATV/r	387 (49)
EFV	400 ^d (51)
Sex, no. (%) of participants	
Female	127 (16)
Male	660 ^d (84)
Race/ethnicity, no. (%) of participants	
White (non-Hispanic)	339 ^d (43)
Black (non-Hispanic)	236 (30)
Hispanic	190 (24)
Asian	14 (2)
American Indian/Alaskan	3 (0.4)
Multiracial	5 (1)
Age (yr), median (Q ₁ , Q ₃) ^b	39 (31, 45)
Wt (kg), median (Q ₁ , Q ₃) ^c	77.3 (68.2, 88.8)
CL _{CR} (ml/min), median (Q ₁ , Q ₃) ^c	113.7 (97.4, 133.6)

^aPercentages are rounded to the nearest whole number; Q₁ and Q₃ represent the 1st and 3rd quartiles, respectively.

^bThree subjects had no age reported, so the mean was used (38.9 years).

^cOne subject had no CL_{CR} or weight data, so the means were used (117.9 ml/min and 79.5 kg, respectively).

^dFour subjects had no race/ethnicity reported and three subjects had no sex or third drug reported, so the mode was used for all three categorical covariates.

The final significant covariates that were identified were creatinine clearance (power model), treatment arm (additive shift; ATV/r as the reference), and race/ethnicity (additive shift; 2 degrees of freedom; white non-Hispanic race as the reference) on apparent clearance (Table 3). The final covariate relationship with apparent clearance was described as follows:

$$CL = \theta_{TVCL} \cdot (CL_{CR}/113.5)^{\theta_{CL_{CR}}} + \theta_{3rd\ drug} \cdot 3rd\ drug + \theta_{RACB} \cdot RACB + \theta_{RACO} \cdot RACO$$

with CL as the apparent total drug clearance, θ_{TVCL} as the estimated typical value of clearance, $CL_{CR}/113.5$ as the creatinine clearance centered to a value of 113.5 ml/min, $\theta_{CL_{CR}}$ as an estimated factor for CL_{CR} , $\theta_{3rd\ drug}$ as the estimated shift in clearance for those in the efavirenz arm compared to those in the atazanavir/r arm, RACB as an indicator variable for black non-Hispanic race/ethnicity compared to white non-Hispanic race/ethnicity, and RACO as the indicator variable for the combination of Hispanic, Asian, American Indian, Alaskan, and multiracial compared to white non-Hispanic.

The final estimated covariate effects showed that there was an average 0.442 change in the natural log of tenofovir plasma CL/F (liters/h) per ml/min in the $\ln CL_{CR}$ compared to the centered value (113.5 ml/min) [e.g., for an individual, $\ln(CL/F) =$

TABLE 2 Pharmacokinetic model development^a

Phase of development	MVOF	IIV CL (% CV)	IIV k_a (% CV)	CCV RV
Base	17,173	23.9		0.0697
R1 FS—CL _{CR}	17,021	20.8		0.0703
R2 FS—CL _{CR} /3rd drug	16,887	18.5		0.0701
R3 FS—CL _{CR} /3rd drug/race or ethnicity	16,860	18.1		0.07
Multivariable—IIV k_a	16,839	17.7	84.5	0.0645
R1 BE	All covariates significant on CL, with addition of IIV on k_a			

^aMVOF, minimum value of the objective function; IIV, interindividual variability; CL, apparent tenofovir plasma clearance; k_a , absorption rate constant; % CV, % coefficient of variation; CCV RV, constant coefficient of variation, residual variability; R1, R2, and R3, round of covariate analysis; FS, forward selection; CL_{CR}, creatinine clearance; 3rd drug, treatment arm (ATV/r versus EFV); BE, backwards elimination.

TABLE 3 Final model parameter estimates for tenofovir pharmacokinetics

Parameter ^e	Model estimate (95% CI)	% RSE ^f (% shrinkage)	Bootstrap estimate ^g (95% CI)
k_a (h^{-1})	2.87 (1.83, 3.91)	18.5	2.87 (2.27, 4.16)
CL/F (liters/h)	37.2 (34.10, 40.29)	4.2	37.40 (33.57, 40.26)
V_c/F (liters)	127 (83.10, 170.90)	17.6	128.73 (86.26, 188.48)
V_p/F (liters)	646 (555.45, 736.55)	7.2	649.55 (547.62, 737.81)
Q/F (liters/h)	107 (70.94, 143.06)	17.2	108.60 (73.26, 144.98)
IIV in k_a	0.714 (0.389, 1.039)	23 (57) ^d	0.817 (0.350, 1.497)
IIV in CL	0.0312 (0.025, 0.037)	10 (17)	0.0309 (0.025, 0.038)
CL _{CR} ^{a,b}	0.442 (0.375, 0.509)	8	0.445 (0.372, 0.511)
Treatment arm ^{a,c}	8 (6.47, 9.53)	10	7.93 (6.51, 9.55)
Black non-Hispanic ^{a,c}	3.17 (1.54, 4.80)	26	3.29 (1.65, 4.90)
"Other" race/ethnicity ^{a,c}	4.09 (2.40, 5.78)	21	4.11 (2.53, 5.84)
CCV RV	0.0645 (0.0576, 0.0714)	5 (16)	0.0641 (0.0575, 0.0712)

^aOn CL/F.

^bPower model.

^cAdditive shift.

^dSignificant ETABAR.

^e k_a , absorption rate constant; CL/F, apparent plasma clearance; V_c/F , apparent volume of distribution in the central compartment. V_p/F , apparent volume of distribution in the peripheral compartment; Q/F, apparent intercompartmental clearance; IIV, interindividual variability; CL_{CR}, creatinine clearance; CCV RV, constant coefficient of variation, residual variability.

^fRSE, relative standard error.

^g960/1,000 = 96% runs contributed; 36 runs with minimization terminated were skipped when calculating the bootstrap results, and 4 runs with estimates near a boundary were skipped when calculating the bootstrap results.

$\ln(37.2 \text{ liters/h}) + 0.442 \cdot \ln(\text{CrCl}/113.5 \text{ ml/min})$]. For the median (113.7 ml/min), first quartile (Q_1) (97.4 ml/min), third quartile (Q_3) (133.6 ml/min), and lowest CL_{CR} (44.4 ml/min) values in the data set, the resultant oral CL values for these individuals would be 37.2, 34.8, 40.0, and 24.6 liters/h, respectively. Those in the efavirenz treatment arm were found to have an average apparent tenofovir clearance that was 8 liters/h greater than that of participants in the atazanavir/ritonavir arm. In comparison to white non-Hispanic individuals, black non-Hispanic participants and those combined into the "other" group (Hispanic, Asian, American Indian/Alaskan, and multiracial) had average apparent tenofovir clearances that were 3.17 liters/h and 4.09 liters/h greater, respectively. A simulation of population predicted values in male participants with median values for weight, CL_{CR}, and age (with no variability) shows the difference in plasma tenofovir exposure between different race/ethnicity groups assigned to either the ATV/r or EFV arms of study A5202 (Fig. 1). Among both treatment arms, those of the "other" and black non-Hispanic race/ethnicity groups were associated with a faster TFV plasma clearance (and therefore reduced plasma exposure) than that of white non-

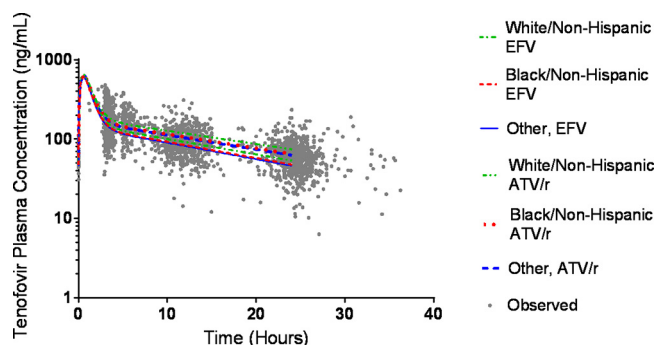


FIG 1 Comparison of predicted TFV concentrations based on race/ethnicity and treatment arm. Simulations are population predictions for males with median values for creatinine clearance, weight, and age. The AUCs (ng · h/ml) were as follows: 2,752 ("other," EFV), 2,804 (black/non-Hispanic, EFV), 3,001 (white/non-Hispanic, EFV), 3,647 (white/non-Hispanic, ATV/r), 3,361 (black/non-Hispanic, ATV/r), and 3,286 ("other," ATV/r).

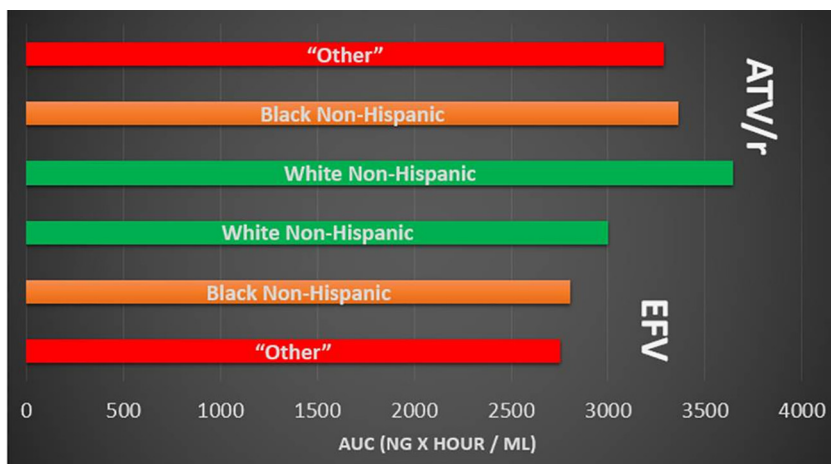


FIG 2 Comparison of TFV plasma exposures in racial/ethnic groups per third drug. AUC, area under the plasma concentration-time curve; EFV, efavirenz; ATV/r, ritonavir-boosted atazanavir; NG, nanogram; ML, milliliter.

Hispanic participants. A comparison of plasma exposures between racial/ethnic groups per third drug treatment arm is provided in Fig. 2.

The estimated mean population pharmacokinetic parameters for the final model, with 95% confidence intervals compared to the bootstrap analysis (96% of runs successfully contributed to the bootstrap analysis and met the predefined convergence criteria), are given in Table 3. The prediction-corrected visual predictive check (pcVPC) was generated with model predictions from 2,000 simulations and shows that the observed median (red line) and 5th and 95th percentiles of the data (blue lines) were included within the model-predicted 95% confidence intervals (shaded areas) (Fig. 3). These internal validation techniques show appropriate model specification.

DISCUSSION

This study has identified CL_{CR} treatment arm, and race/ethnicity as significant covariates associated with apparent clearance following the recommended TDF dose in treatment-naïve adults living with HIV. Tenofovir disoproxil fumarate is recommended as a first-line nucleotide reverse transcriptase inhibitor in national HIV treatment guidelines (7), and its use has increased among non-HIV-infected individuals for pre-exposure prophylaxis, when used with emtricitabine (8, 9). These findings may provide additional considerations for individualizing TDF-based regimens.

Multiple structural population pharmacokinetic models for plasma tenofovir have been reported as two-compartment models with first-order absorption and elimination (4, 5, 10, 11). Previous population analyses (3–5, 10, 11) have identified plasma tenofovir pharmacokinetic parameters in the following ranges: CL/F , 42 to 66.6 liters/h, apparent volume of distribution in the central compartment (V_c/F), 268 to 1,040 liters; apparent intercompartmental clearance (Q/F), 13.2 to 197 liters/h; apparent volume of distribution in the peripheral compartment (V_p/F), 398 to 1,630 liters; and k_{el} , 0.82 to 1.35 h^{-1} . These values were similar to those estimated in the current analysis: CL/F , 37.2 liters/h; V_c/F , 127 liters; Q/F , 107 liters/h; V_p/F , 646 liters; and k_{el} , 2.87 h^{-1} . The difference noted in the absorption rate constant may be due to differences in population administration of tenofovir in relation to food, as food delays the time to tenofovir maximum concentration in serum (C_{max}) (1). Previous pharmacokinetic studies of TFV have also identified renal function and ritonavir-enhanced protease inhibitors as significant covariates in the plasma exposure of TFV (3–5, 10, 11). Our analysis confirms that ATV/r in combination with TDF results in increased TFV plasma concentrations compared to coadministration with EFV. In a study of 12 participants listed in the prescribing information for atazanavir, there was a 37% (90% confidence interval [CI], 1.30 to 1.45)

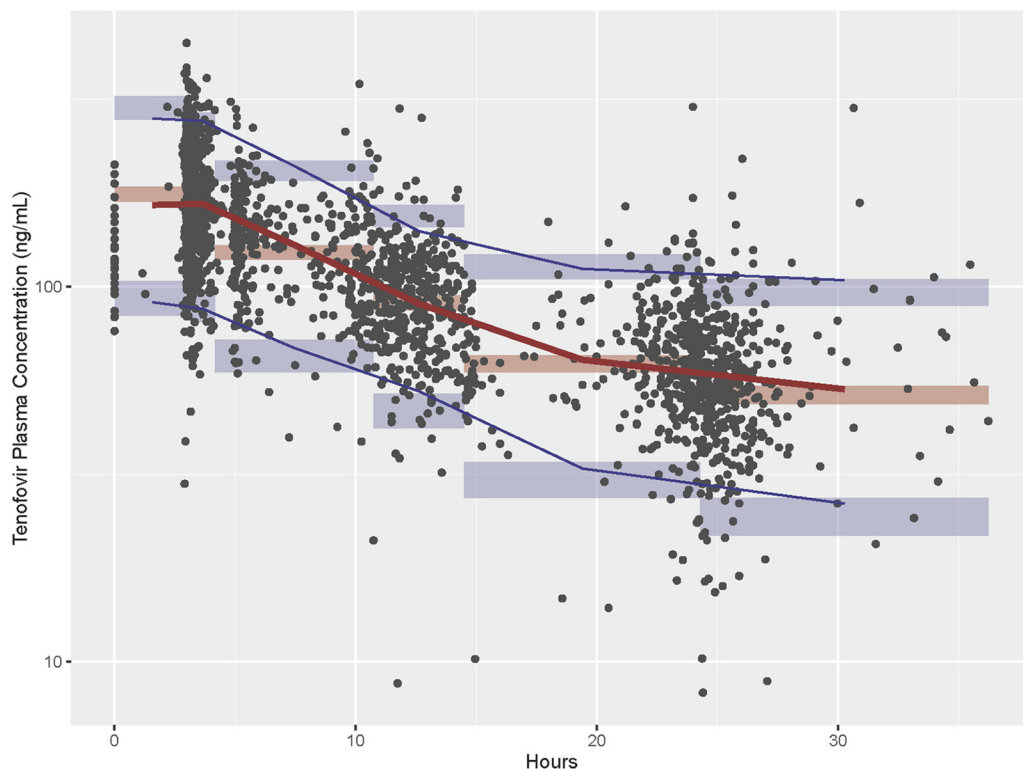


FIG 3 Prediction-corrected visual predictive check comparing observed and model predicted drug concentrations. Observed concentrations and their 5th, 50th, and 95th percentiles are overlaid on the simulated 95% confidence intervals of the corresponding percentiles.

increase in the TFV area under the concentration-time curve (AUC) when coadministered with ATV/r (12).

Study 5202 was a multicenter clinical trial across the United States, including Puerto Rico, which enrolled a large number of participants, including well-represented minority populations. Approximately 23% ($n = 429$) of the study population were Hispanic, and 33% ($n = 615$) were black non-Hispanic, allowing for the evaluation of the pharmacokinetics of TFV in these patient populations. Among the three race/ethnicity groups evaluated (white non-Hispanic versus black non-Hispanic and versus “other”), there was a significant difference in TFV oral CL when the white non-Hispanic group was compared to the other two groups. To our knowledge, this is the first report to identify race/ethnicity as a significant covariate for TFV oral CL. The “other” race/ethnicity group in this study consisted predominantly of participants who were Hispanic (190 Hispanic participants out of 212 in this group). Individuals in this race/ethnicity group were associated with a faster TFV oral clearance, compared to white non-Hispanic participants, and therefore reduced plasma exposure (Fig. 1). The question arises if a difference in clearance could affect adverse effects over time. ACTG study A5224s was a substudy of A5202 ($n = 269$) focused on assessing longer-term changes in metabolic outcomes in participants treated with FTC/TDF or ABC/3TC with either EFV or ATV/r. A5224s compared changes in areas such as limb fat, renal function, glucose, insulin and insulin sensitivity, urine albumin, and urine protein/creatinine ratios between different race/ethnicity groups. However, information comparing these groups within the TDF/FTC arms to the ABC/3TC arms (13–16) was not reported. In the univariate analysis, there was no significant association between race/ethnicity (compared to white non-Hispanic participants) with changes in body mass and bone mineral density adjusted for treatment arm (17).

In the iPrEx trial evaluating oral daily doses of TDF/FTC or placebo as preexposure prophylaxis in HIV-seronegative men or transgender women who have sex with men,

a relative risk reduction from contracting HIV of 92% was found in those with detectable drug concentrations (quantification range, 10 to 1,500 ng/ml) in the treatment arm (8). The majority of the iPrEx study population was Hispanic (approximately 72%), with the largest number of participants from Peru; however, a separate analysis of ARV exposures by race/ethnicity has not been reported.

Limitations of the current analysis include unknown reasons for variability of drug concentrations, such as other potential drug-drug interactions, effects of comorbid conditions, or other unmeasured confounding factors. In addition, the observations identified by the residual variability mixture model represent apparent nonadherence, since only 2 of the 78 observations identified by the mixture model reported slight deviations from daily dosing (2 days between the second and third dose prior to pharmacokinetic sampling). Unreported noncompliance may be one possible source of the large residual variability seen within the data.

In conclusion, a population pharmacokinetic model with covariate analysis was developed for ACTG study A5202 to analyze the TFV plasma concentration data available from the diverse participant population. The significant covariates associated with apparent TFV clearance included creatinine clearance, treatment arm, and race/ethnicity. As the number of low- and middle-income countries are moving toward antiretroviral regimens containing dolutegravir (DTG) as the preferred first-line treatment, including the fixed-dose combination of TDF/3TC/DTG (18), the findings of this analysis are timely and relevant. These data suggest that additional research examining preexposure prophylaxis regimens and use for treatment may consider possible TFV exposure differences between race/ethnicity groups.

MATERIALS AND METHODS

Participants and study design. Study A5202 was a phase 3b, randomized, partially blinded equivalence study of four once-daily ARV regimens in treatment-naïve adults (≥ 16 years of age). Participants were randomized 1:1:1:1 and balanced by study site. At screening, study participants were stratified by HIV-1 RNA level ($< 100,000$ or $\geq 100,000$ copies/ml). Participants in the high-screening viral load stratum (based on data safety monitoring board recommendations) and those with toxicity associated with the NRTI backbone who had virologic failure or hepatitis B were unblinded. The primary efficacy, safety, and tolerability results have been reported (19–21).

A sparse sampling strategy for the measurement of plasma drug concentrations was designed to collect three steady-state samples per participant during the first 24 weeks of therapy, including dosing information on the previous 4 doses prior to sampling. Pharmacokinetic drug concentration sampling occurred at week 4, 8, 16, or 24. Three samples were to be collected over two visits, visits A and B. Visit A could occur before or after visit B, or the visits could be combined if medications were regularly scheduled for the morning. At visit A, two plasma samples were obtained, one before a dose and one 3 to 4 h after an observed dose; at visit B, one sample was to be obtained 5 to 15 h (5 to 12 h if all medications were taken in the morning) after a dose (22–24).

Assurances. The human subject committees of all sites approved the A5202 protocol, and written informed consent was obtained from all participants in compliance with human experimentation guidelines of the U.S. Department of Health and Human Services.

Tenofovir sample processing. Plasma concentrations of tenofovir were measured at the ACTG Pharmacology Laboratory at the University of Alabama at Birmingham using a high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS) assay (25). After the addition of stably labeled isotope internal standards (IS), samples were processed using a solid-phase extraction method on 30-mg (1-cm³) Oasis MCX cartridges. The eluate was then evaporated under a nitrogen stream and reconstituted in 175 μ l of 0.01% trifluoroacetic acid (TFA) for injection. Using a Shimadzu XR HPLC system, reversed phase chromatographic separation of the drug and the internal standard was performed on an Atlantis dC₁₈ analytical column (2.1 by 100 mm, 3.0 μ m) under isocratic conditions. A Supelco C₁₈ in-line guard cartridge was used to protect the column from contaminants. A column temperature of 30°C, a rate of 0.2 ml/min, and an injection volume of 10 μ l were used. The binary mobile phase consisted of 0.1% formic acid (A) and 0.1% formic acid (B) in acetonitrile with a composition of 90% A to 10% B. Under these conditions, the retention time for tenofovir and its respective internal standard was approximately 2.12 min. A total run time of 5 min was used to ensure complete elution of all peaks of interest. Detection and quantification were accomplished on an API 5500 mass spectrometer by multireaction monitoring. Tenofovir and the IS were detected using the following transitions for protonated products $[M + H]^+$: m/z 288.2 $>$ 176.1 for tenofovir; m/z 293.2 $>$ 181.0 for tenofovir-IS. Mass spectrometer settings were as follows: an API 5500 instrument was used in positive TubolonSpray mode with a source temperature of 500°C. Nitrogen was used as the nebulizer, auxiliary, collision, and curtain gas. The calibration curve was linear over the range of 5 to 1,000 ng/ml for tenofovir using a 50- μ l aliquot of human plasma. Validation of the plasma assay showed precision with less than 8% coefficient of variation between calibration standards on different assay runs and accuracy with less than 6% deviation

from known concentrations. Plasma quality controls showed overall precision [(SD/mean) × 100%] with less than 7% and accuracy [(mean – target)/target × 100%] within 10% CV.

Population pharmacokinetic modeling. An exploratory pharmacokinetic analysis identified significant differences in TFV oral CL when participant characteristics for A5202 were compared (22). With this previously identified structure as a starting base model, the goal of the current analysis was to identify and quantify sources of variability in TFV exposure, while confirming and refining the exploratory base structural model. Pharmacokinetic analysis of TFV (136-mg dose) was performed using nonlinear mixed-effects modeling with NONMEM (version 7.1.0; ICON Development Solutions, Ellicott City, MD, USA) interfaced with Perl-speaks-NONMEM (PsN; <https://uopharmacometrics.github.io/PsN/>) through Pirana (version 2.9.4; <http://www.pirana-software.com/>) as the modeling environment (26, 27). The first-order conditional estimation method with interaction was used with a steady-state assumption. One- and two-compartment models with first-order absorption and first-order elimination were evaluated. An exponential error model with log-normal distribution was used for examination of intersubject variability of all pharmacokinetic parameters, adding to the intersubject variability on CL/F which was already contained in the exploratory model. A proportional and mixed (additive and proportional) error model was assessed for residual variability on one- and two-compartment models. In addition, a mixture model of residual variability was used to detect apparent nonadherence during the model building process (28). Goodness-of-fit plots were used to evaluate the base structural model: observations versus individual and population predictions (IPRED and PRED), conditional weighted residuals versus PRED and time, and absolute individual weighted residuals versus IPRED.

Covariate selection. A covariate analysis was performed to explore the association of several factors with plasma TFV pharmacokinetic parameters. Race/ethnicity, sex, and third drug treatment arm (ATV/r or EFV) were evaluated as categorical covariates using an additive functional form. Race/ethnicity groups available for model building included white non-Hispanic, black non-Hispanic, Hispanic, Asian, American Indian/Alaskan, and multiracial (although the sample size was small for the last three groups). Each group was evaluated individually and combined in stepwise increments starting with those groups with the smallest number of participants. Continuous covariates, which included age at study entry, creatinine clearance (CL_{CR}; calculated by the Cockcroft-Gault equation) (29), and weight in kilograms, were centered and evaluated using power and linear functional forms. Weight and CL_{CR} measurements were obtained at the same time or in close proximity to the time points for tenofovir sampling. Significant covariates were selected using the classical stepwise approach (30). Forward selection was conducted in a univariate manner, with covariate inclusion into the model being determined by a reduction in minimum value of the objective function (MVOF) by >3.84 ($P < 0.05$; 1 degree of freedom, in a χ^2 distribution) and by a reduction in the interindividual variability (IIV) of the pharmacokinetic parameter of interest. For the backward elimination step, covariates were removed separately and considered significant if their removal resulted in an increase in the MVOF of at least 10.83 ($\alpha < 0.001$; 1 degree of freedom, in a χ^2 distribution). Time-varying covariates (CL_{CR} and weight) were included in the data set if CL_{CR} changed to a different stage of renal function (table can be found in the Food and Drug Administration's "Guidance for Industry on Pharmacokinetics in Patients with Impaired Renal Function" [31]) and weight if it changed by >20 lb. Between forward selection and backward elimination, multivariable model evaluation occurred by exploring the addition of interindividual variability on additional parameters and examination of the residual variability model by precision.

Model evaluation. The final model was evaluated using 2,000 simulations in a prediction-corrected visual predictive check (pcVPC). Observed concentrations and their 5th, 50th, and 95th percentiles were overlaid on the 95% confidence interval of the corresponding percentiles of the simulations for visual assessment. Prediction correction was used to take into account the differences within a bin coming from the included covariates (32). All the observations and predictions were then normalized using the typical population prediction in relation to the median of typical population predictions per bin across the covariates (32). Nonparametric bootstrap resampling was also performed to evaluate the model (33). One thousand bootstrap data sets were generated to obtain a median and 95% confidence interval for each model parameter for comparison with the original final parameter estimates, with an *a priori* convergence criterion set at 90% when modeling the bootstrap data sets for evaluation of the convergence rate.

ACKNOWLEDGMENTS

We thank the study participants for their generous donation of time and effort in the successful completion of clinical trial A5202. We also thank Olivia Campagne (University at Buffalo, SUNY) for advice on population modeling and Katie Mollan (University of North Carolina, UNC) for advice and statistical contributions to ACTG A5202. We also acknowledge contributions, either financial and/or drug related, made by GSK/ViiV Healthcare, Gilead, Bristol-Myers Squibb, and Abbott (AbbVie) to the parent study (A5202) and/or substudies of A5202.

Other investigators and contributors included the following: Hector H. Bolivar and Sandra Navarro, University of Miami (site 901), CTU grant AI069477, ACTG grant AI27675, and CFAR grant AI073961; Susan L. Koletar and Diane Gochnour, Ohio State University (site 2301), CTU grant AI069474; Edward Seefried and Julie Hoffman, University of California, San Diego (site 701), CTU grant AI69432; Judith Feinberg and Michelle Saemann, University of Cincinnati (site 2401), CTU grant AI069513; Kristine

Patterson, Donna Pittard, and David Currin, University of North Carolina (site 3201), CTU grant AI69423, CFAR grant AI50410, GCRC grant RR00046, and grant RR025747; Kerry Upton and Michael Saag, University of Alabama (site 5801), CTU grant U01 AI069452, CCTS grant 1UL1 RR025777-01; Graham Ray and Steven Johnson, University of Colorado Health Sciences Center (site 6101), CTU grant AI69450, grant AI054907, and grant RR00051; Bartolo Santos and Connie A. Funk, University of Southern California (site 1201), CTU grant 5U01 AI069428; Michael Morgan and Brenda Jackson, Vanderbilt Therapeutics CRS (site 3652), CTU grant AI069439; Pablo Tebas and Aleshia Thomas, University of Pennsylvania, subunit of Children's Hospital of Philadelphia (site 6201), CTU grant U01 AI069467-03, CFAR grant 5P30 AI045008-10; Ge-Youl Kim and Michael K. Klebert, Washington University (site 2101), CTU grant AI069495; Jorge L. Santana and Santiago Marrero, University of Puerto Rico (site 5401), CTU grant 5U01 AI069415-03; Jane Norris and Sandra Valle, Stanford University (site 501), CTU grant AI69556; Gary Matthew Cox and Martha Silberman, Duke University Medical Center (site 1601), CTU grant 5U01 AI069484-02; Sadia Shaik and Ruben Lopez, Harbor-University of California, Los Angeles Medical Center (site 603), CTU grant AI069424; Margie Vasquez and Demetre Daskalakis, New York University/NYC HHC at Bellevue Hospital Center (site 401), CTU grant AI069532; Christina Megill and Todd Stroberg, Cornell Chelsea (site 7804), CTU grant AI69419, CSTC grant RR024996; Jessica Shore and Babafemi Taiwo, Northwestern University CRS (site 2701), CTU grant AI069471; Mitchell Goldman and Molly Boston, Indiana University (site 2601), CTU grant UO1 AI025859; Jeffrey Lennox and Carlos del Rio, Ponce de Leon Center (A5802), CTU grant 5U01 AI069418, CFAR grant P30AI050409; Timothy W. Lane and Kim Epperson, Moses H. Cone Memorial Hospital (site 3203), CTU grant 1U01 AI069423-01; Annie Luetkemeyer and Mary Payne, University of California, San Francisco (site 801), CTU grant 1U01 AI069502-01; Barbara Gripshover and Dawn Antosh, Case Western Reserve University (site 2501), CTU grant AI69501; Jane Reid and Mary Adams, University of Rochester (site 1101), CTU grant U01 AI069511, GCRC grant UL1 RR024160; Sheryl S. Storey and Shelia B. Dunaway, University of Washington (site 1401), CTU grant AI069434; Joel Gallant and Ilene Wiggins, Johns Hopkins University (site 201), CTU grant AI69465; Kimberly Y. Smith and Joan A. Swiatek, Rush University Medical Center (site 2702), CTU grant 5U01 AI069471; Joseph Timpone and Princy Kumar, Georgetown University (site 1008), CTU grant 1U01 AI069494-01; Ardis Moe and Maria Palmer, University of California, Los Angeles Care Center (site 601), CTU grant AI069424; Jon Gothing and Joanne Delaney, Brigham and Women's Hospital, Boston, MA (site 107), CTU grant AI069472; Kim Whitely and Ann Marie Anderson, Metro Health Center (site 2503), CTU grant AI069501; Scott M. Hammer and Michael T. Yin, HIV Prevention & Treatment (Columbia University) (site 30329), CTU grant 5U01 AI069470, grant 1UL1 RR024156; Mamta Jain and Tianna Petersen, UT Southwestern Medical Center at Dallas (site 3751), CTU grant 3U01 AI046376 05S4; Roberto Corales and Christine Hurley, AIDS Community Health Center (site 1108), CTU grant U01AI069511, GCRC grant UL1 RR024160; Keith Henry and Bette Bordenave, Hennepin County Medical Center (site 1502), grant N01 AI72626; Amanda Youmans and Mary Albrecht, Beth Israel Deaconess (Partners/Harvard) CRS (site 103), CTU grant UOI AI06947203; Richard B. Pollard and Abimbola Olusanya, University of California, Davis Medical Center (site 3851), grant AI38858; Paul R. Skolnik and Betsy Adams, Boston Medical Center CRS (site 104), CTU grant AI069472; Karen T. Tashima and Helen Patterson, Miriam Hospital-Brown University (Partners/Harvard) (site 2951), CTU grant 1U01 AI069472-01; Michelle Ukwu and Lauren Rogers, Peabody Health Center (site 31443), CTU grant AI069471; Henry H. Balfour, Jr., and Kathy A. Fox, University of Minnesota (site 1501), CTU grant AI27661; Susan Swindells and Frances Van Meter, University of Nebraska Medical Center (site 1505), CTU grant AI27661; University of Hawaii (site 5201), CTU grant AI34853; Gregory Robbins and Nicole Burgett-Yandow, Massachusetts General Hospital from the Partners/Harvard/BMC ACTU (site 101), CTU grant 1U01 AI069472-01; Charles E. Davis, Jr., and Colleen Boyce, IHV Baltimore Treatment CRS (site 4651), CTU grant 5U01 AI069447 03; William A. O'Brien and Gerianne Casey, University of Texas Medical Branch (site 6301), CTU grant AI032782; Gene D. Morse and Chiu-Bin Hsaio, SUNY-Buffalo (site

1102), CTU grant 5U01 A1027658; San Mateo County AIDS Program (site 505), CTU grant A127666; Jeffrey L. Meier and Jack T. Stapleton, University of Iowa Healthcare (site 1504), NIAID grant A127661, grant A158740; Donna Mildvan and Manuel Revuelta, Beth Israel Medical Center ACTU (site 2851), CTU grant A146370; David Currin, Wake County HHS (site 30076), CTU grant A125868; Wafaa El Sadr and Avelino Loquere, Harlem ACTG CRS (site 31483), CTU grant 5U01 A1069470-03; Nyef El-Daher and Tina Johnson, McCree McCuller Wellness Center (site 1107), CTU grant U01 A1069511, GCRC grant UL1 RR024160; Robert Gross and Kathryn Maffei, University of Pennsylvania Health (site 6206), CTU grant 1U01 A169467-01; Valery Hughes and Glenn Sturge, Cornell Uptown (site 7803), CTU grant 1U01 A1069419-01; Deborah McMahon and Barbara Rutecki, University of Pittsburgh (site 1001), CTU grant 1U01 A1069494-01; Michael Wulfsohn, Andrew Cheng, and Norbert Bischofberger, Gilead Sciences; and Lynn Dix and Qiming Liao, GlaxoSmithKline, Inc.

Research reported in this publication was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award numbers UM1 A1068634, UM1 A1068636, UM1 A1069424, UM1 A1069511, and UM1 A1106701. Research was supported in part by the University of Rochester Center for AIDS Research under award number P30 A1078498, the University of Washington/Fred Hutchinson Cancer Research Center for AIDS Research under award number UM1 A127757, the University of California San Francisco Pharmacology Core under award number P30 A1022763, the University of North Carolina Center for AIDS Research under award number P30 A1050410, and the University at Buffalo Pharmacology Specialty Laboratory and the University of Alabama Pharmacology Specialty Laboratory under award number UM1 A1106701. C. S. Venuto and Q. Ma were supported in part by grants K23AI108355 and K08MH098794, respectively.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

E.S.D.'s institution receives grant support from Gilead, Merck, and ViiV, and E.S.D. is a consultant for Bristol Myers Squibb, Gilead, Merck, Janssen, Teva, and ViiV. A.C.C. is a member of a Data and Safety Monitoring Board for a Merck-sponsored study, and her institution has received grant support from Bristol-Myers-Squibb. K.Y.S. is an employee of ViiV Healthcare, beginning December 2013. All other authors have no conflict of interest to declare.

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