


ORIGINAL ARTICLE

Population pharmacokinetics of tenofovir, emtricitabine and intracellular metabolites in transgender women

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Aims: Transgender women (TGW) have been underrepresented in trials and use gender-affirming hormonal therapies (GAHT) that may alter renal function by significantly increasing creatinine clearance. Population pharmacokinetic (popPK) models and simulations would aid in understanding potential differences in emtricitabine/tenofovir disproxil fumarate (F/TDF) parent-metabolite concentrations in TGW on GAHT when compared to cisgender men (CGM) not exposed to GAHT.

Methods: Pharmacokinetic (PK) data from a Phase 1, open-label clinical trial with directly observed therapy of daily F/TDF consisting of 8 TGW and 8 CGM was utilized for model building. PopPK analysis was performed using nonlinear mixed effects modelling (NONMEM 7.5.0). Covariates of body weight, creatinine clearance, and gender were evaluated. Final models were subjected to Monte Carlo simulations to compare drug exposure following once daily and on-demand (IPERGAY 2 + 1 + 1) dosing of F/TDF.

Results: Tenofovir (TFV) and emtricitabine PK were best described by a 2-compartment model, first-order absorption/elimination with absorption lag time. Parent models were linked to their metabolites by first order formation and elimination. Creatinine clearance was a significant covariate influencing clearance in both models. Simulations demonstrated that at least 2, weekly 2 + 1 + 1 cycles of on-demand dosing in TGW on GAHT is necessary for TFV-diphosphate to reach similar exposure after the initial week of on-demand dosing in CGM not on GAHT.

Conclusion: PopPK models of TFV, emtricitabine and intracellular metabolites in TGW were established. Dose simulations revealed that TGW should be treated for at least 2 weeks to have comparable exposures to CGM.

KEYWORDS

HIV, popPK model, population pharmacokinetics, pre-exposure prophylaxis, tenofovir, transgender women

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1 | INTRODUCTION

Transgender women (TGW) remain at high-risk for HIV infection, with an estimated global prevalence of 19%; they are 49-times more likely to acquire HIV than cisgender women of reproductive age.¹ TGW are a key population that would benefit from pre-exposure prophylaxis (PrEP) for HIV prevention. However, there are barriers to PrEP uptake, including access to care, social stigma and patient concerns regarding the impact of PrEP on gender-affirming hormonal therapies (GAHT).^{2–4} TGW frequently access oestrogen-based GAHT to induce physical changes and promote congruence with their gender identity; these therapies may also include anti-androgenic agents.^{5,6} The World Professional Association for Transgender Health has published transgender-focused recommendations to promote feminization and reduce gender dysphoria in TGW.⁷ There is also heterogeneity in dosages and formulations of GAHT accessed by TGW. Given the variety in GAHT options available to TGW, in conjunction with existing concerns regarding unfavourable interactions between GAHT and PrEP regimens, more research is required in this area.

Daily oral tenofovir disoproxil fumarate (TDF) 300 mg/emtricitabine (FTC) 200 mg (F/TDF) was the first drug regimen approved by the US Food and Drug Administration for PrEP. Daily product use was shown to be highly effective in reducing HIV acquisition; subsequent studies showed that an alternative *on-demand* (IPERGAY 2 + 1 + 1) PrEP regimen in men who have sex with men is associated with an 86% relative risk reduction in HIV infection.^{8–10} Earlier trials evaluating the efficacy of F/TDF demonstrated decreased PrEP efficacy and lower adherence in TGW in comparison to cisgender men (CGM) who have sex with men.⁹ However, the relationship between F/TDF, and their active metabolites, tenofovir-diphosphate (TFV-DP) and emtricitabine-triphosphate (FTC-TP), and GAHT, is not completely understood. Two studies reported 24–32% lower TFV and FTC plasma concentrations in TGW when compared to contemporaneously studied CGM.^{11,12} However, Shieh *et al.* did not identify statistically significant differences in TFV-DP concentrations in peripheral blood mononuclear cells (PBMC) between populations¹²; a similar finding was observed in a more recent study, which showed comparable intraerythrocytic TFV-DP concentrations collected as dried blood spots between CGM and TGW on stable GAHT.¹³ Further, neither study found an impact of PrEP on GAHT.¹² However, due to the variety and variability of analytical methods, as well as heterogeneity of GAHT regimens accessed among these studies, additional efforts to comprehensively assess the impact of GAHT on oral F/TDF for PrEP are warranted.

Population pharmacokinetic (popPK) modelling is used to describe sources of PK parameter variability as well as their covariate relationships. PopPK models can facilitate clinical trial design, dose selection, and dose timing optimization. Numerous popPK studies describe the relationship between plasma TFV or FTC and PBMC TFV-DP or FTC-TP concentrations.^{14–19} However, none of these studies include TGW on GAHT. In this report, we developed a joint parent–metabolite model to characterize the interrelationship of plasma TFV/FTC with PBMC TFV-DP/FTC-TP in non-HIV-infected TGW and CGM based

What is already known about this subject

- Transgender women (TGW) remain at high-risk for HIV infection.
- The relationship between emtricitabine/tenofovir disoproxil fumarate (F/TDF) and gender-affirming hormonal therapy (GAHT) is not completely understood.
- Studies have shown lower plasma emtricitabine and tenofovir concentrations in TGW when compared to cisgender men (CGM).

What this study adds

- A joint F/TDF parent–metabolite population pharmacokinetic model in TGW on GAHT and CGM demonstrate that drug concentrations are influenced by renal function.
- Simulations indicate that on-demand F/TDF requires 2 weekly 2 + 1 + 1 cycles in TGW on GAHT to achieve comparable tenofovir-metabolite concentrations observed during a single 2 + 1 + 1 cycle in CGM.

on data first reported in a descriptive noncompartmental analysis from our group.¹² We then used the model to simulate a comparison of daily and on-demand F/TDF PrEP in TGW on GAHT.

2 | METHODS

2.1 | Trial design

The clinical study generating the data utilized in the popPK models and assay performance characteristics are described elsewhere.¹² This analysis was approved by the North Texas Regional Institutional Review Board and the Johns Hopkins Medicine Institutional Review Board. Study data were collected between April 2016 and April 2018, and all participants provided informed consent to participate in the clinical study.¹² Briefly, 8 TGW and 8 CGM were enrolled in a phase 1, open-label PK study of once daily TDF 300 mg/FTC 200 mg under direct observation in a research clinic for 8 days. Study participants (CGM and TGW) were required to have a baseline creatinine clearance (CrCl) ≥ 70 mL/min, using the Cockcroft–Gault formula for inclusion in the clinical trial. Additional inclusion criteria for the protocol required TGW to be on oestrogen-based GAHT with serum total oestradiol concentration >100 pg/mL. GAHT consisting of oestrogen \pm progesterone or \pm antiandrogen medication were used by TGW in this cohort. After 7 days of directly observed dosing, blood was collected predose, and 1, 2, 4, 6, 8 and 24 hours postdose, and PBMC were collected predose, and 2, 8 and 24 hours postdose.

All doses of TDF and FTC and drug analyte concentrations were converted to micromolar units for comodelling. The intracellular metabolite concentrations were converted to micromolar units using 282 fL/cell as cellular volume.²⁰ The molecular weights used for conversion were: TDF, 635.52 g/mol; TFV, 287.216 g/mol; and FTC, 247.248 g/mol.

2.2 | PopPK model development

PopPK analysis was performed using NONMEM (version 7.5.0, ICON Development Solution, Ellicott City, MD, USA) with Perl speaks NONMEM (PsN version 4.9.0) as an interface. R software (version 4.0.3) was used for dataset preparation and graphics. PopPK data analyses and subsequent simulations were performed between June 2020 and April 2021.

Parent and metabolites were modelled simultaneously, in which plasma drug disposition was evaluated first, then the PBMC compartment was added to describe metabolite concentrations. One- and 2- compartment models with first-order absorption, with and without a lag time, were tested for structural models. Metabolite compartments were linked to a first-order formation rate constant once a reasonable parent model was established. An exponential error model was used to describe interindividual variability, which was assumed to be normally distributed with mean of zero and variance of ω^2 . Additional information on the popPK models may be found in Supplemental File 1.

For each analyte, additive, proportional, and combined additive and proportional residual variability models were assessed separately, and were assumed to be normally distributed with mean of zero and variance of σ^2 .

Model selection was driven by a global evaluation based on NONMEM objective function value (OFV), the Akaike information criteria (AIC), successful convergence, goodness-of-fit (GOF) plots, and precision and biological plausibility of parameter estimates. A decrease in the objective function by 3.84 units ($P < .05$, 1 degree of freedom) for hierarchical models was considered to be significant. For non-nested model comparisons, the AIC was used for model selection using the following formula: $AIC = NONMEM\ OFV + 2 \times \text{number of parameters in the model}$. A drop in AIC of 2 or more was a threshold for considering 1 model over another.²¹

For covariate screening, we used biological rationale, parameter vs. covariate scatterplots for continuous variables, and boxplots for categorical variables. Body weight, CrCl (estimated by Cockcroft-Gault equation using the female term for TGW), and self-identified gender were evaluated for the impact on PK parameters. Continuous covariates were modeled using a median-normalized power model. Categorical covariate was modeled using a proportional model. We used forward inclusion and backward elimination procedures along with the likelihood ratio test to evaluate the effect of each covariate on model parameters. Covariates were considered significant and included in the basic model if the decrease in OFV was >3.84 units

($P < .05$, 1 degree of freedom) for forward inclusion, and the increase in OFV was >6.64 units ($P < .01$, 1 degree of freedom) for backward elimination.²²

2.3 | Model evaluation

GOF plots, visual predictive checks (VPCs),²³ and nonparametric bootstrap analysis were utilized to evaluate the final models and parameter estimates.²⁴ GOF plots include individual (IPRED) and population (PRED) predictions vs. observed concentrations, and conditional weighted residuals (CWRES) vs. time after dose and PRED.²⁵ VPCs were conducted using PsN and R. A total of 500 replicates of the simulated dataset were produced from the final models and used for the determination of prediction intervals. The 5th, 50th and 95th percentiles of the simulated concentrations were plotted against the time after dose and compared with observed TFV, FTC, TFV-DP and FTC-TP. The nonparametric bootstrap method was performed to evaluate reliability and stability of the final models. Nonparametric bootstraps of 500 iterations were conducted to estimate 95% confidence intervals (CIs) for the population parameters.

2.4 | Monte Carlo simulations to compare on-demand vs. QD dosing regimen

Monte Carlo simulations of plasma (TFV and FTC) and PBMC (TFV-DP and FTC-TP) were performed for 500 virtual subjects with gender specific CrCl using the final population PK models. For gender-specific CrCl, random samples ($n = 500$) of CrCl were generated from mean and standard deviation from the trial data using R. Four different dosing scenarios were simulated with respect to a sexual activity (potential exposure of virus) as a reference time point:

Scenario 1: Subjects were on conventional daily dosing (F/TDF) before sexual exposure and at steady state, consistent with Food and Drug Administration label dosing

Scenario 2: Subjects initiate conventional daily dosing on the day of sexual exposure to represent initial phase of conventional daily dosing

Scenario 3: On-demand dosing in which subjects took a double dose 2 hours before exposure followed by a single dose 24 hours after the first dose, and a single dose 24 hours after the second dose (1 cycle), consistent with the *on-demand* IPERGAY 2 + 1 + 1 regimen. On-demand dosing is interchangeably referred to as 2 + 1 + 1 regimen in the remaining text.

Scenario 4: Same as Scenario 3 but repeat the regimen for a total of 2 weeks (2 cycles)

For each simulated scenario, a mean concentration-time profile and boxplot of C_{24} (trough) concentrations for TFV/FTC/TFV-DP/FTC-TP after the exposure were compared.

3 | RESULTS

3.1 | Data summary

Demographic summary of the participants is shown in Table 1. A total of 179 concentration values from 16 subjects were available for the model building exercise. Most participants were Black (75%). TGW showed no differences in age or weight compared to CGM ($P > .05$). There were statistically significant differences in serum creatinine and CrCl between genders ($P < .05$), with TGW showing 24% lower creatinine and 61% higher CrCl when compared to CGM (Figure S1). These observations were not accounted for by a statistically nonsignificant 18% weight difference ($P > .05$). No analyte concentrations were below the limit of quantification.

3.2 | PopPK model of FTC and FTC-TP

Comparison of 1- and 2-compartment models for plasma FTC using AIC and OFV confirmed that a 2-compartment model provided a better fit and significantly smaller objective function (ΔOFV of -107.8 and ΔAIC of -101.8 for FTC). With statistical criteria as well as GOF plots, FTC was best described by a 2-compartment model with first-order absorption and elimination. The addition of absorption lag time further reduced the objective function (ΔOFV of 33.0 for FTC) and improved the fit of the observations in the absorption phase. It was found that parent FTC was best linked with intracellular FTC-TP by first-order formation and elimination. Residual error was modelled using a proportional error model. CrCl calculated using female correction factor for TGW was shown to be a significant covariate on clearance (ΔOFV of -11.7). Gender and CrCl were colinear. Based on biological plausibility, CrCl was included in the model. In a sequential step, gender was tested as a covariate in addition to CrCl, but was not a significant covariate. Inclusion of CrCl as a covariate reduced the between-subject variability (BSV; %CV) on CL to 7.5% from 12.9% in the base model. A schematic of the model is shown in Figure 1. The intracellular half-life for FTC-TP was calculated as 15 hours.

TABLE 1 Demographics of the participants

	TGW (n = 8)	CGM (n = 8)	P value
Age, y ^a	29 (27–38)	46 (38–51)	.195 ^b
Weight, kg ^a	98 (85–113)	83 (73–89)	.130
CrCl (mL/min) ^a	174 (117–184)	108 (85–121)	.038
SCr (mg/dL) ^a	0.80 (0.80–0.83)	1.05 (1.00–1.12)	.005
Race			>.999 ^c
African ancestry	6	6	
European ancestry	1	2	
Asian ancestry	1	0	

^aData expressed as median (interquartile range).

^bExact 2-sided P value, Wilcoxon rank sum test.

^cFisher's exact test.

Abbreviations: CGM, cisgender men; CrCl, creatinine clearance; SCr, serum creatinine; TGW, transgender women.

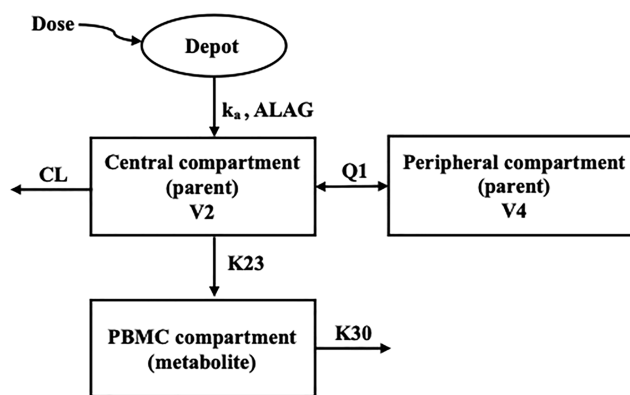


FIGURE 1 Model schematic for tenofovir disoproxil fumarate and emtricitabine disposition. k_a , absorption rate constant; ALAG, lag time; CL, clearance from the central compartment; V2, central compartment volume of distribution; V4, peripheral compartment volume of distribution; Q1, intercompartmental clearance; K23, formation rate constant of metabolite stimulated by plasma drug; K30, elimination rate constant of intracellular metabolite

The percent relative standard error (%RSE) of the PK parameter estimates was acceptable with $<30\%$ for fixed effect parameters and residual error, and $<65\%$ for random effects. The shrinkage was $<30\%$ for all random effects parameters that were estimated on absorption rate constant, except BSV (37% shrinkage). GOF plots of the final model are shown in Figure S2. These plots showed no major bias and were considered acceptable. The VPC plot represented in Figure S3 indicated a good predictive performance of the model showing an agreement between the distribution of observed and predicted data. Final parameters along with bootstrap 95% confidence intervals (CIs) are provided in Table 2.

3.3 | PopPK model of TFV and TFV-DP

A decrease in objective function and AIC, and an improvement in GOF plots were observed in plasma TFV when comparing 1- and 2-compartment models (ΔOFV of -133.9 and ΔAIC of -127.9 for TFV). A 2-compartment model with first-order absorption best described plasma TFV concentration. After TFV-DP was linked to the plasma compartment with the first-order formation by the simultaneous estimation approach, higher interindividual variability, RSE and correlation of the parameters (K23 - K32 and K30 - ALAG with 0.95 and 0.90, respectively) were observed. The shrinkage was $<30\%$ for all random effects parameters that were estimated. The implementation of nonlinear metabolite formation using the indirect response model approach¹⁶ to describe plasma TFV and PBMC TFV-DP concentrations failed to converge. The sequential estimation approach showed similar PK parameter estimates when compared to the simultaneous approach. Gender and CrCl were significant covariates on CL when testing with a forward inclusion step (ΔOFV of -5.2 for gender and ΔOFV of -10.2 for CrCl). Following backward elimination, CrCl was a significant covariate of CL. Inclusion of CrCl as a covariate

TABLE 2 Emtricitabine and emtricitabine triphosphate population pharmacokinetic parameter estimates for the final models and bootstrap results for the final model

Parameter (unit)	Final model Estimate (% RSE)	Bootstrap Median (95% CI)
<i>Fixed effects</i>		
k_a (1/h)	3.10 (20)	2.95 (1.09–32.32)
V2 (L)	76.1 (6)	71.7 (51.7–83.4)
V4 (L)	77.9 (13)	70.5 (55.6–108.1)
Q1 (L/h)	7.89 (12)	9.07 (5.95–15.22)
CL (L/h)	19.8 (3)	19.2 (16.7–21.4)
ALAG (h)	0.522 (26)	0.464 (0.005–0.979)
K30 (1/h)	$4.69E^{-2}$ (13)	$4.78E^{-2}$ ($1.82E^{-2}$ – $8.50E^{-2}$)
K23 (1/h)	$1.08E^{-2}$ (15)	$1.16E^{-2}$ ($4.20E^{-3}$ – $2.96E^{-2}$)
<i>Covariate effect</i>		
CrCl on CL*	$2.96E^{-2}$ (18)	$3.12E^{-1}$ ($1.76E^{-1}$ – $4.63E^{-1}$)
<i>Interindividual variability (%CV)</i>		
k_a	139.6 (33)	112.1 (36.5–235.6)
ALAG	65.3 (65)	78.0 (0.7–361.8)
Q1	42.3 (53)	27.3 (0.4–68.9)
CL	7.5 (51)	4.5 (0.1–12.4)
K30	28.5 (34)	25.8 (7.3–36.0)
<i>Residual variability (% CV)</i>		
Proportional error on parent	18.3 (18)	17.9 (13.5–22.7)
Proportional error on metabolite	43.4 (29)	42.6 (30.3–56.7)

$$*CL_{\text{individual}} = CL_{\text{population}} \times \left(\frac{CrCl_{\text{individual}}}{CrCl_{\text{median}}} \right)^{CrCl_{\text{onCL}}} \times e^{BSV_{CrCl}}$$

Abbreviations: ALAG, lag time; CL, clearance; CrCl, creatinine clearance; K23, formation rate of constant of metabolite stimulated by plasma drug; K30, elimination rate constant of intracellular metabolite; k_a , absorption rate constant; Q1, intercompartmental clearance; V2, central compartment volume of distribution; V4, peripheral compartment volume of distribution.

reduced the BSV (%CV) on CL to 18.6% from 31.2% in the base model. Residual error was modeled using a proportional error model. Final parameters along with 95% bootstrap CIs are provided in Table 3. A schematic of the model is shown in Figure 1. The intracellular half-life for TFV-DP was estimated as 173 hours.

GOF plots of the final model are shown in Figure S4. These plots showed no major bias. The VPC plot represented in Figure S5 indicated a good predictive performance of the model showing an agreement between the distribution of observed and predicted data. Final parameters along with bootstrap 95% confidence intervals are provided in Table 3.

3.4 | Simulations of the conventional daily dose and on-demand dosing

Simulated continuous concentration–time profiles (Figure 2) and discrete C_{max} and C_{min} concentrations on day 3 and 10 (Table S1) for TFV, TFV-DP, FTC, and FTC-TP contrasted differences between daily and on-demand PrEP, as well as differences between TGW and CGM. At all times over 2 weeks, PBMC TFV-DP concentrations

after on-demand dosing were below median steady-state concentrations with daily dosing consistent with expectations for 4 vs. 7 doses per week (18.8 vs. 36.3 fmol/ 10^6 cells); this was also observed for PBMC FTC-TP, although with far smaller differences (4 doses: 0.6 pmol/ 10^6 cells; 7 doses: 2.2 pmol/ 10^6 cells). The highest trough (C_{min}) and peak (C_{max}) PBMC TFV-DP concentrations during on-demand dosing were 44 and 52% of daily dosing concentrations in week 1 (occurring on day 3) and increased to 63 and 67% of daily dosing concentrations in week 2 (occurring on day 10) and thereafter, with pseudo-steady-state achieved in week 2. Contrasting TGW and CGM with on demand dosing, the highest trough and peak PBMC TFV-DP concentrations in TGW were 83 and 84% of CGM during weeks 1 and 2. In TGW on an on-demand regimen, the highest trough and peak PBMC TFV-DP concentrations were 38 and 43% of daily dosing steady-state concentrations in CGM during the first week (day 3), and improved to 53 and 55% in week 2 (day 10) and thereafter. With a far shorter half-life, the highest trough concentrations of PBMC FTC-TP in all weeks of on-demand dosing were similar to steady-state daily dosing; on-demand dosing in TGW yielded PBMC FTC-TP concentrations only 8% below CGM on the same on-demand regimen.

TABLE 3 Tenofovir and tenofovir diphosphate population pharmacokinetic parameter estimates for the final models and bootstrap results for the final model

Parameter (unit)	Final model Estimate (% RSE)	Bootstrap Median (95% CI)
<i>Fixed effects</i>		
k_a (1/h)	3 FIXED	3 FIXED
V2 (L)	234 (38)	234 (74–332)
V4 (L)	657 (92)	656 (489–848)
Q1 (L/h)	227 (27)	227 (136–298)
CL (L/h)	50 (13)	50 (43–54)
ALAG (h)	0.290 (123)	0.289 (0.003–0.403)
K30 (1/h)	$9.14E^{-3}$ (303)	$8.94E^{-3}$ ($9.14E^{-5}$ – $1.17E^{-2}$)
K23 (1/h)	$2.03E^{-5}$ (309)	$2.03E^{-5}$ ($2.00E^{-7}$ – $4.52E^{-5}$)
<i>Covariate effect</i>		
CrCl on CL*	$5.97E^{-1}$ (72)	$5.89E^{-1}$ ($1.50E^{-1}$ – $8.74E^{-1}$)
<i>Interindividual variability (%CV)</i>		
k_a	130.8 (789)	130.5 (39.9–167.7)
CL	18.6 (142)	18.6 (10.5–23.1)
V2	22.6 (238)	22.1 (0.5–31.6)
V4	42.5 (155)	41.5 (3.7–47.0)
ALAG	114.0 (522)	113.8 (77.2–307.3)
<i>Residual variability (% CV)</i>		
Proportional error on parent	15.7 (27)	15.7 (11.7–18.3)
Proportional error on metabolite	37.1 (70)	37.1 (26.6–45.3)

$$*CL_{\text{individual}} = CL_{\text{population}} \times \left(\frac{CrCl_{\text{individual}}}{CrCl_{\text{median}}} \right)^{CrCl_{\text{onCL}}} \times e^{BSV_{CrCl}}$$

Abbreviations: ALAG, lag time; CL, clearance; CrCl, creatinine clearance; K23, formation rate of constant of metabolite stimulated by plasma drug; K30, elimination rate constant of intracellular metabolite; k_a , absorption rate constant; Q1, intercompartmental clearance; V2, central compartment volume of distribution; V4, peripheral compartment volume of distribution.

4 | DISCUSSION

This report describes the generation of an F/TDF popPK model that specifically focuses on TGW and the application of drug exposure simulations that take into consideration different populations and dosing regimens. Further, these simulations reflect current, on-demand modalities for F/TDF PrEP use. This work focuses on an at-risk population and aims to determine optimal dosing strategies in TGW to ensure drug exposures consistent with protection.

We developed a joint parent–metabolite popPK model describing plasma TFV/FTC and intracellular TFV-DP/FTC-TP based on both TGW on GAHT and CGM, which demonstrated the substantial influence of CrCl variability on drug clearance for both TFV and FTC. Our parameter estimate for the CrCl effect on drug clearance is generally in agreement with previous studies.^{14,16,26–28} Because gender was associated with CrCl, and based on biological rationale of the influence of hormones on renal function, we included only CrCl in the final model.

The use of the 0.85 correction factor recommended for females in the Cockcroft–Gault CrCl calculation results in a 15% decrease in calculated CrCl in TGW when compared to CGM. In the phase 1 clinical trial used for model generation, median CrCl was 174 mL/min and

108 mL/min in TGW and CGM, respectively. This represents a 61% increase in CrCl in TGW, which is not accounted for by the Cockcroft–Gault female correction factor, or the statistically insignificant 15% higher median body weight that can also influence CrCl. As CrCl was calculated using the 0.85 multiplier for TGW, it is expected that the model parameters estimates would need to be re-estimated if any other multiplier is used. However, we anticipate that the conclusions of the simulations would remain similar. More direct measurement of glomerular filtration rate using other methods, such as iohexol clearance, would provide a more accurate covariate of TFV and FTC renal clearance.

A 2-compartment model with first order absorption and elimination best described TFV plasma PK. Final parameters are in general agreement with previous reports for TFV.^{14–19,27} Metabolite formation rate constant (K23) is in reasonable agreement with our prior report, once we take into consideration the difference in units for metabolite compartment.¹⁴ The elimination half-life for the metabolite estimate (calculated as $0.693/K30$) from this report is higher than Burns *et al.*,¹⁴ but consistent with prior publications, which ranged from 150 to 180 hours.^{29–31} Potential sources of such variability across studies may include sampling time differences and limits of quantification used in metabolite measurements.

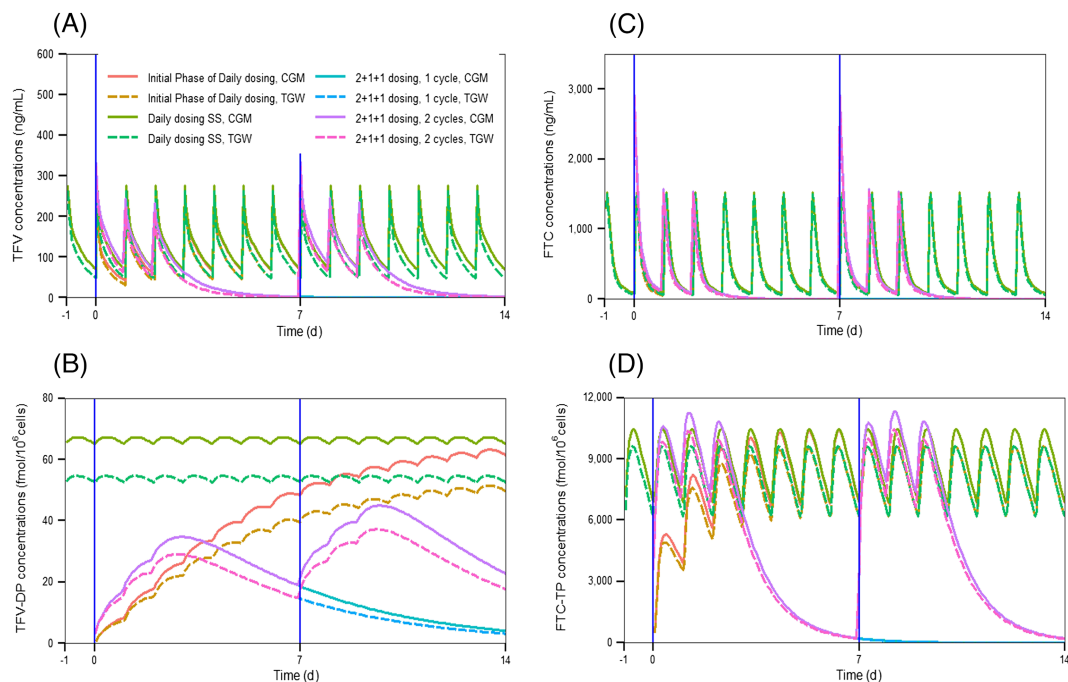


FIGURE 2 Simulated mean concentration–time profiles of (a) tenofovir (TFV), (b) TFV-diphosphate (TFV-DP), (c) emtricitabine (FTC) and (d) FTC-triphosphate (FTC-TP) on conventional daily dose and 2 + 1 + 1 dosing. Blue line represents exposure point. CGM, cisgender men; TGW, transgender women

Plasma FTC was best described as a 2-compartment model with first order absorption and elimination. PK parameter estimates are in general agreement with previous reports.^{17–19,28,32} Saturable functions for metabolite formation were not used, given only 1 dose level. The previous elimination half-life of FTC-TP was 17–36 hours^{33,34}; however, a slightly shorter half-life of FTC-TP of 15 hours was observed in this study.

Several studies have shown that the use of F/TDF for PrEP with GAHT is associated with decreased plasma TFV/FTC concentrations.^{11,12,35} Hiransuthikul *et al.* reported 12 and 18% reductions in AUC and trough concentration of plasma TFV in TGW using GAHT; however, the study did not evaluate plasma FTC or intracellular metabolite concentrations.¹¹ Cirrioncione *et al.* reported a reduction of 24 and 14% AUC in plasma TFV and FTC, respectively, in TGW compared to historical controls; however, they reported significantly higher (>2-fold higher) PBMC concentrations of metabolites in TGW.³⁵ Shieh *et al.* evaluated both plasma TFV/FTC along with their intracellular components. They reported a reduction of 27 and 24% in AUC for plasma TFV and FTC concentrations in TGW compared to CGM, respectively. A reduction of 24 and 12% in AUC for TFV-DP and FTC-TP, respectively, was reported in this study, although the observation was not statistically significant.¹² The clinical data described by Shieh and colleagues were used to generate the models described in this manuscript.

We conducted simulations to compare daily dosing and on-demand dosing strategies as well as TGW and CGM in terms of TFV, FTC, TFV-DP, and FTC-TP exposure. As expected, the combination of fewer doses per week and the days long PBMC TFV-DP half-life

resulted in lower concentrations with on-demand dosing throughout the simulated dosing periods. Comparing the highest PBMC TFV-DP concentrations during the initial (week 1) 4-dose on-demand F/TDF regimen, TGW and CGM achieved only 45 and 53% of the lowest steady-state daily dosing concentrations in CGM. In the second week and thereafter (at pseudo-steady-state), peak on-demand dosing TFV-DP concentrations increased to 57 and 69%; notably, PBMC TFV-DP concentration in TGW during the second week of a weekly on-demand regimen catches up with CGM concentrations achieved in the first on-demand dosing week. The IPERGAY and PREVENIR trials studied on-demand 2 + 1 + 1 F/TDF dosing with most participants taking the regimen weekly. Both studies demonstrated low to non-existent instances of PrEP failure.⁸ In the original IPERGAY study, only a small number of participants reported taking the on-demand regimen less than weekly, but there was no apparent difference in PrEP efficacy in this group.⁸ In addition, because there are no studies of an on demand 2 + 1 + 1 regimen with lower F/TDF doses than those used in the largely CGM populations in IPERGAY and PREVENIR, we previously raised concern that the 24%–32% reduction in plasma TFV concentrations^{11,12} could reduce the efficacy of on-demand regimens, though have no negative impact on daily regimens. Combined with the simulations performed here, this provides some level of reassurance that, at least in the second week of on-demand dosing, F/TDF protection in TGW is likely to be as high as in the initial on-demand week in CGM, despite the observed impact of GAHT on TFV and TFV-DP concentrations.¹² This is further reinforced by the simulations indicating minor differences in FTC-TP across gender and dosing conditions. An essential caveat, however, is that neither the relative

importance of peaks or troughs on any given day of the on-demand regimen, nor the relative contribution of TFV-DP and FTC-TP with the F/TDF fixed dose combination, are established.

Our models have several limitations. First, poor precision was observed in parameter estimates due to the small dataset used for model building. Second, our models assume linear PK and simulations conducted in terms of accumulation of exposure. Third, the sampling times for TFV-DP in the study do not ensure true steady-state conditions; therefore, this limits the accumulation ratio estimate; however, we do not believe this limitation impacts the overall findings of the described work. Extrapolation of models beyond this PrEP formulation is not ideal and should be carefully considered. This present study serves as an important starting point to explore an alternative regimen for TGW to help clinical study designs. Further validation of models is required before clinical implementation for alternative dosing decisions. Lastly, TFV alafenamide, which has very different blood and tissue TFV-DP PK when compared to TDF, requires its own models and simulations.

5 | CONCLUSIONS

Joint popPK models for describing TFV/FTC and TFV-DP/FTC-TP concentrations after orally administered F/TDF in TGW have been developed and described in this report. Simulations indicate the on-demand regimen requires 2 weekly 2 + 1 + 1 cycles in TGW on GAHT to ensure achievement of similar PBMC TFV-DP concentrations observed during the initial 2 + 1 + 1 regimen in CGM. Further, the 2 + 1 + 1 regimen in CGM, proven highly effective as PrEP, protects with only 1/2 to 2/3 of the TFV-DP concentrations associated with daily dosing.

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COMPETING INTEREST

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CONTRIBUTORS

C.W.H., E.J.G., E.S., R.P.B. and M.A.M. performed the original research study. A.T., A.C., C.W.H. and M.A.M. designed this research study. A.T. and A.C. developed the models and performed simulations. A.T., A.C., C.W.H. and M.A.M. drafted and edited manuscript. All authors read successive versions of the manuscript and approved the final manuscript.

STUDY PI DECLARATION

The authors confirm that the PI for this paper is Mark Marzinke, PhD. The authors also claim that the PI for the clinical study in which these data are based upon is Craig Hendrix, MD, and that he had direct clinical responsibility for patients.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study may be available from the corresponding author upon reasonable request and in compliance with relevant institutional policies.

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SUPPORTING INFORMATION

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