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Clinical trial simulation to evaluate tenofovir disoproxil fumarate/emtricitabine HIV pre-exposure prophylaxis dosing during pregnancy

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Objective: To evaluate upward-adjustment of tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC) pre-exposure prophylaxis (PrEP) dosing during pregnancy in order to maintain target plasma concentrations associated with HIV protection.

Design: Population pharmacokinetic (PK) modeling and clinical trial simulation (CTS).

Material and methods: We developed population pharmacokinetic models for TFV and FTC using data from the Partners Demonstration Project and a PK study of TDF/FTC among cisgender women by Coleman et al., and performed an in-silico simulation. Pregnancy-trimester was identified as a significant covariate on apparent clearance in the optimized final model. We simulated 1,000 pregnant individuals starting standard daily oral TDF/FTC (300 mg/200 mg) prior to pregnancy. Upon becoming pregnant, simulated patients were split into two study arms: one continuing standard-dose and the other receiving double standard-dose throughout pregnancy.

Results: Standard-dose trough TFV concentrations were significantly lower in pregnancy compared to pre-pregnancy, with 34.0%, 43.8%, and 65.1% of trough plasma concentrations below the lower bound of expected trough concentrations presumed to be the protective threshold in the 1st, 2nd, and 3rd trimesters, respectively. By comparison, in the simulated double-dose group, 10.7%, 14.4%, and 27.8% of trough concentrations fell below the estimated protective thresholds in the 1st, 2nd, and 3rd trimesters, respectively. The FTC trough plasma concentration during pregnancy was also lower than pre-pregnancy, with 45.2% of the steady-state trough concentrations below the estimated protective trough concentrations of FTC. In the pregnancy-adjusted double-dose group, 24.1% of trough plasma concentrations were lower than protective levels.

Conclusions: Our simulation shows >50% of research participants on standard dosing would have 3rd trimester trough plasma TFV concentrations below levels associated with protection. This simulation provides the quantitative basis for the design of prospective TDF/FTC studies during pregnancy to evaluate the safety and appropriateness of pregnancy-adjusted dosing.

KEYWORDS

pregnancy, pre-exposure prophylaxis, HIV infection, tenofovir, emtricitabine, clinical trial simulation, population pharmacokinetic modeling

Introduction

Pre-exposure prophylaxis (PrEP) is critically important for the prevention of Human Immunodeficiency Virus (HIV) during pregnancy, both for prevention of maternal HIV and secondary perinatal transmission. Oral tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) is the most commonly used PrEP medication for people with receptive vaginal exposure to HIV and has extensive safety data in pregnancy; however, dosing and efficacy have not been prospectively evaluated in pregnancy. Multiple studies of TDF/FTC during pregnancy both for treatment and prevention of HIV report lower tenofovir (TFV) exposures in the 2nd and 3rd trimesters attributed to pregnancy-related increased volume of distribution and renal clearance (1–16). Similar declines in FTC concentrations are also reported (7–9, 11). The Partners Demonstration Project showed the largest decline during pregnancy compared to non-pregnant women, with 45%–58% reductions in plasma TFV and intraerythrocytic TFV diphosphate (TFV-DP) concentrations from dried blood spots, respectively, compared to non-pregnant women (1). Decreases in peripheral blood mononuclear cell (PBMC) TFV-DP concentrations of up to 49% were also reported (1). Additionally, although plasma TFV concentrations are 20%–25% higher during the first 6 weeks postpartum than in the 3rd trimester, they remain lower than non-pregnant concentrations (4, 5). Lower TFV exposure during pregnancy is of particular concern, as meta-analyses, pooled study analyses, and pharmacometric modeling studies indicate that non-pregnant women already require higher drug concentrations required to achieve high levels of HIV protection in women compared to men (17–21). While plasma and PBMC concentrations of parent drugs (TFV, FTC) and active anabolites (TFV-DP, FTC-TP), respectively, are the same in men and women, drug deposition and TFV-DP concentrations are lower in cervicovaginal tissue as compared to colorectal tissue, which may contribute to the differences in TDF/FTC efficacy between men who have sex with men (MSM) vs. women (22–28).

We hypothesized that without doubling the TDF/FTC dose in pregnancy, substantial losses in HIV protection of 20%–40% would be expected due to moving down the concentration-response curve (17, 18, 29). The objective of the current analysis is to evaluate the effect of pregnancy on the pharmacokinetics (PK) of TDF and FTC in a population pharmacokinetics (popPK) modeling framework using a nonlinear mixed effects approach and to perform a clinical trial simulation to evaluate the appropriateness of a pregnancy-adjusted double TDF/FTC dose. Since the majority of TDF is rapidly converted to TFV after oral absorption, TFV is the primary circulating form of the drug in the plasma (30); thus the modeling and simulation were based on TFV plasma concentrations.

Materials and methods

Study design and study data

This analysis utilized popPK models of TFV and FTC and clinical trial simulation to compare the adequacy of standard

TDF/FTC dosing to a pregnancy-adjusted, double TDF/FTC dose to maintain target plasma concentrations associated with HIV protection in the 1st, 2nd and 3rd trimesters of pregnancy. The pregnancy-adjusted double-dose TDF/FTC regimen was selected based on the demonstrated pregnancy-related concentration decreases in both TFV and FTC reported in the PK literature (1–9, 11–13, 15, 16).

We included data from two studies in the popPK modeling: the Partners Demonstration Project and data from the TDF/FTC arm of a phase I, prospective, open-label study conducted in Baltimore, Maryland by Coleman and colleagues (31, 32). The Partners Demonstration Project was a multi-site, randomized, double-blind, placebo-controlled clinical trial conducted in Kenya and Uganda, which included PK data from 116 female participants, including 33 pregnant and postpartum participants who became pregnant while taking TDF/FTC and elected to continue on TDF/FTC. TDF/FTC was provided in a MEMS[®] container, which records a time-and-date stamp for each container opening as a proxy for medication ingestion. The Coleman study included intensively sampled, steady-state PK data from 12 non-pregnant, pre-menopausal, HIV negative, cisgender women taking TDF/FTC under directly observed therapy (DOT). We chose the Partners Demonstration Project as it sampled the largest published cohort of pregnant and postpartum individuals on TDF/FTC PrEP. We included the Coleman, et al., PK study to supplement the Partners Demonstration Project PK data with intensive PK data under DOT. For both studies, plasma TFV and FTC concentrations were measured using a previously described, validated liquid chromatographic-tandem mass spectrometric (LC-MS/MS) assay (27). Lower limits of quantification (LLOQ) for plasma TFV and FTC were 0.31 ng/ml. All plasma drug concentrations were measured by the Clinical Pharmacology Analytical Laboratory at the Johns Hopkins University School of Medicine.

Dataset preparation

We prepared the datasets for modeling by integrating MEMS data on adherence and TFV/FTC concentration data from the Partners Demonstration Project and dosing records from the Coleman et al. study. We used “M3” method articulated by Beal to handle drug concentrations below the limit of quantification (BLQ) in the Partner Demonstration Project. The M3 method accounts for measurements BLQ explicitly without censoring them. Thus, these observations are included in the PK model analysis using an appropriate statistical approach (33).

Modeling and simulation

We conducted the population analysis using NONMEM (version 7.3. ICON Development Solution, USA) with the gfortran compiler interfaced with Perl-speaks-NONMEM (PsN). Dataset preparation and diagnostic plot plotting were carried out using R (4.1.1). The clinical simulation was carried out using mrgsolve package (1.0.8) in R.

Model development

We developed the base model for TFV and FTC using the data from the Coleman et al. study. Based on the published models, we tested one-compartment and two-compartment models with first-order absorption and with or without lag time. After the development of the base model, we simultaneously used data from both the Coleman et al. study and the Partners Demonstration Project study for parameter estimation. For TFV, the exponential between subject variability was supported on first order absorption rate constant (Ka), apparent clearance (CL/F), apparent central (Vc/F) and peripheral volumes (Vp/F), and apparent inter-compartmental clearance (Q/F); For FTC, the exponential between subject variability was supported on Ka, CL/F, Vp/F, and Q/F:

$$P = TVP \cdot \exp(\eta_p) \quad \eta_p \sim N(0, \omega_p^2)$$

Where the P represents the individual value of the parameter P , the TVP represent the typical value of the parameter P , the η_p denotes the inter-individual variability (IIV) which is assumed to have a normal distribution with mean equals to 0 and variance equals to ω_p^2 .

For both TFV and FTC, we used a proportional residual model for the Coleman et al. study and a combined residual model for the Partners Demonstration Project to account for the heterogeneity of two clinical trials:

$$C_{ij} = \widehat{C}_{ij} \cdot (1 + \varepsilon_{1ij} \cdot (2 - \text{STUDY}) + \varepsilon_{2ij} \cdot (\text{STUDY} - 1)) \\ + \varepsilon_{3ij} \cdot (\text{STUDY} - 1) \\ \varepsilon_{1ij} \sim N(0, \sigma_1^2), \varepsilon_{2ij} \sim N(0, \sigma_2^2), \text{ and } \varepsilon_{3ij} \sim N(0, \sigma_3^2)$$

Where the C_{ij} represents the observed concentration of subject i at time j , the \widehat{C}_{ij} represents the predicted concentration, STUDY represents the study number (i.e., 1—Coleman et al. study, 2—Partners Demonstration Project). ε_{1ij} and ε_{2ij} represent the proportional error of data from the Coleman, et al. and the Partners Demonstration Project studies. ε_{3ij} represents the additive error of data from the Partner Demonstration Project.

Covariate evaluation

We tested potential covariates for TFV and FTC parameters, independently, using study number (i.e., 1 or 2 as above), baseline creatinine clearance, and pregnancy status. We treated pregnancy status as a categorical variable using 4 categories (0—non-pregnant, 1—1st trimester, 2—2nd trimester, and 3—3rd trimester). We evaluated different grouping methods on pregnancy data to test if the influence of each trimester could be identified separately. The pregnancy data were grouped as 1st trimester vs. 2nd trimester vs. 3rd trimester, 1st trimester and 2nd trimester vs. 3rd trimester, 1st trimester vs. 2nd trimester and 3rd trimester. Aggregation of all trimesters as a single factor was also tested. To assess covariate relationships, we first visualized the empirical Bayes estimates versus the potential covariates, and then employed stepwise selection method. For the forward selection, a decrease of the OFV more than

3.84 was considered significant for one degree of freedom ($p < 0.05$). For the backward elimination, an increase of OFV more than 6.63 was considered significant for one degree of freedom ($p < 0.01$).

Model evaluation

We evaluated the performance of the final model by the diagnostic plots. This included evaluating the conditional weighted residuals and review of visual predictive checks. Concentrations associated with extreme deviations from the model prediction were assessed individually for physiologic plausibility. If an appropriate explanation of the outlier was not identified, the outlier was removed. A prediction corrected visual predictive check (pcVPC) of the final model showed the 5th, 50th, and 95th predicted percentiles from 1,000 simulated datasets with 128 individuals (12 from the Coleman et al. study and 116 from the Partners Demonstration Project), and generated the observed concentrations of TFV and FTC. The simulated concentrations that were BLQ were truncated to the LLOQ (0.31 ng/ml). We stratified the VPC by study.

Clinical trial simulation

Based on the selected final population pharmacokinetic model, we conducted a clinical trial simulation to evaluate trough concentrations (C_{trough}) of TFV and FTC during pregnancy. We simulated PK profiles of 1,000 cisgender female participants taking standard daily oral 300 mg TDF/200 mg FTC prior to pregnancy. Upon becoming pregnant, simulated participants were split into two arms: arm 1 ($n = 500$) continuing the standard dose regimen and arm 2 ($n = 500$) receiving a pregnancy-adjusted, double-dose of both TFV and FTC. We assumed an increase in renal clearance due to pregnancy beginning in the 1st trimester. Simulated trough plasma concentrations of TFV and FTC were compared with the lower bound of expected trough concentration benchmarks, estimated to be the protective thresholds associated with daily dosing estimated from HPTN 066, 35.5 ng/ml for TFV and 49.1 ng/ml for FTC (28).

Results

The final dataset included data from 128 women (12 from the Coleman, et al., study and 116 from the Partners Demonstration Project; see **Table 1**). Data included 33 pregnant women, of whom, 29, 24, and 23 women contributed data from their 1st, 2nd, and 3rd trimesters, respectively. For TFV, there are 39 (6 BLQ) samples in the 1st trimester, 59 (14 BLQ) samples in the 2nd trimester, and 62 (20 BLQ) samples in the 3rd trimester. For FTC, there are 37 (9 BLQ) samples in the 1st trimester, 55 (15 BLQ) samples in the 2nd trimester, and 55 (22 BLQ) samples in the 3rd trimester. Total concentrations available for modeling included 487 TFV and 465 FTC measurements. Upon visual exploration of the final model, outliers were noted in the pcVPC. Further examination revealed four TFV measurements (0.82% of the total measures) and twelve

TABLE 1 Participant demographics from the partners demonstration project and the coleman et al. study.

Parameter	Partners demonstration		Coleman et al.
	Non-pregnant	Pregnant	Non-pregnant
Number of participants	97 (83 with plasma samples)	37 (33 with plasma samples)	12
Race ^a	–	–	9B, 2W, 1A
Ethnicity ^a	–	–	1H
	mean (SD)		median (IQR)
Age	30.6 (7.4)	25.1 (4.8)	34 (28–37)
Weight (kg)	–	–	90 (78–101)
BMI	24.5 (4.3)	24.6 (4.7)	–
CrCl (ml/min)	101.8 (18.4)	111.7 (27.5)	139 (115–172)
iGFR (ml/min/1.73 m ²)			102 (88–114)

^aBlack (B), White (W), Asian (A), Hispanic (H).

FTC measurements (2.6% of total measures) that were physiologically implausible. These were removed from the dataset and the population model re-run. Minor differences were noted for TFV in the CL (51.5 vs. 52.4 L/h) and Vp/F (1,160 L vs. 1,120 L). A larger change was observed in the Vc/F (359 vs. 252 L).

In the FTC population model, CL did not change; however, the central volume of distribution changed from 90.7 to 66.9 L and the peripheral volume of distribution changed from 195 to 166 L.

TFV model

A two-compartment model with first order absorption adequately described the pharmacokinetics of TFV in this population (Table 2); the diagnostic plots and VPCs indicated good agreement between observed and predicted values

TABLE 2 Final estimates of TFV pharmacokinetic parameters, between subject variability, and residual variability^a.

Parameter	Estimate	RSE%
CL/F (L/h)	52.4	7
V2/F (L)	252	57
Q/F (L/h)	295	18
V3/F (L/h)	1120	18
KA (/h)	2.56	91
CL/F increment during 1st trimester (%)	21.4%	55
CL/F increment during 2nd trimester (%)	33.9%	34
CL/F increment during 3rd trimester (%)	63.9%	29
BSV on CL/F	35.9%	13
BSV on V2/F	41.4%	145
BSV on Q/F	67.6%	29
BSV on V3/F	56.7%	28
BSV on KA	56.4%	87
σ1(prop; Coleman study)	21.2%	6
σ2(prop; partner demonstration project)	71.2%	5
σ2(add; partner demonstration project) (ng/ml)	0.109	253

^aCL/F, apparent clearance; V2/F, apparent volume of distribution of the central compartment; Q/F, apparent intercompartmental clearance; V3/F, apparent volume of distribution of the peripheral compartment; KA, absorption rate constant; BSV, between-subject variability; σ1, proportional residual error; σ2, additive residual error; RSE, relative standard error.

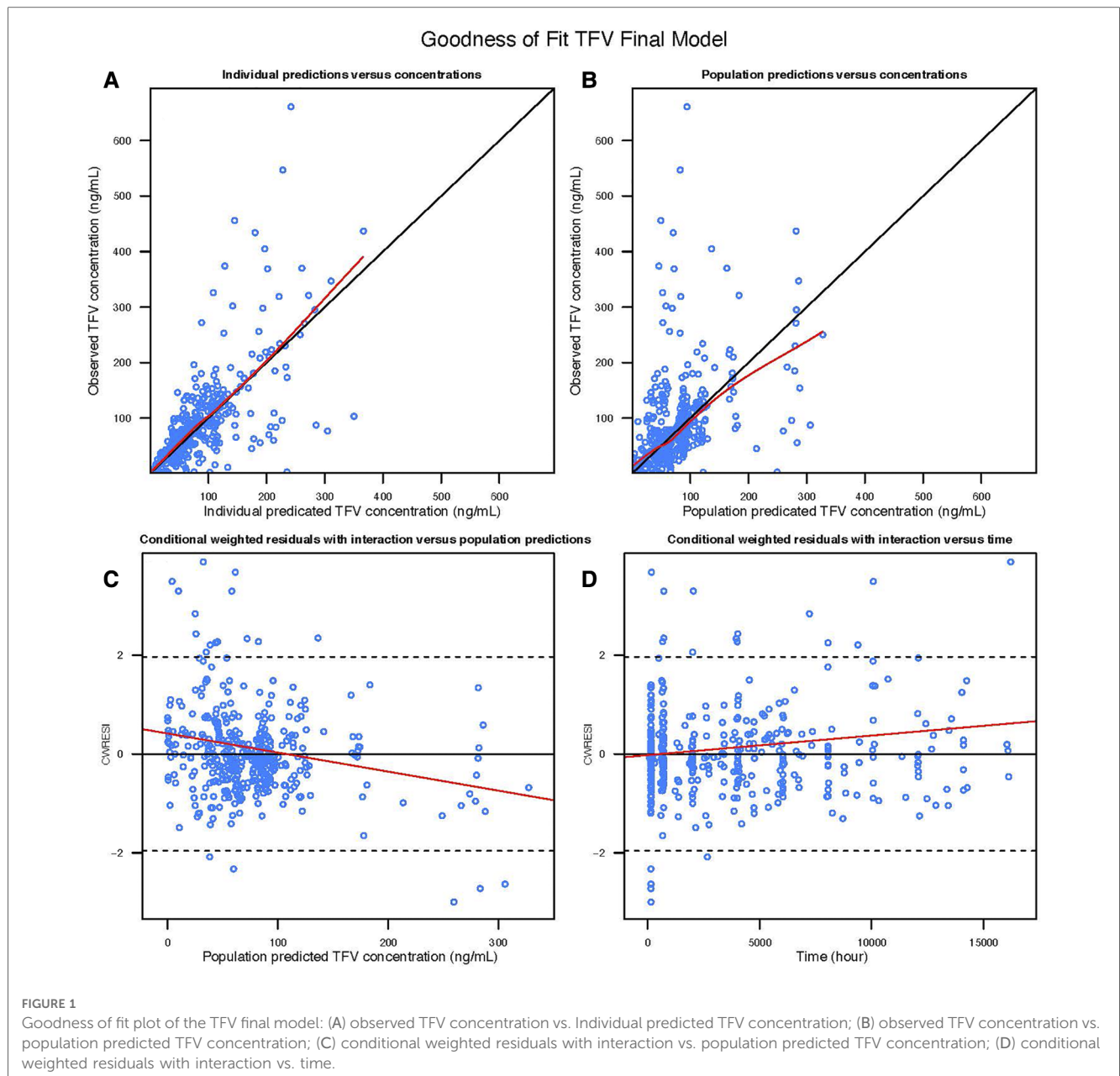
(Figures 1, 2). Our final model overestimated the TFV trough concentration in the Coleman et al. study, as seen in Figure 2. Inclusion of trimester as a covariate in the apparent clearance significantly reduced the objective function value (OFV) by 48.809. The typical value of the apparent clearance of TFV increased by 1.214, 1.339, and 1.639-fold in the 1st, 2nd, and 3rd trimester, respectively, compared to the non-pregnant baseline values; these data are comparable to the previously reported clearance increment during each trimester. The proportional error of the Partners Demonstration Project (71.2%) was higher than the Coleman et al. study (21.2%).

FTC model

We selected a two-compartment model with first-order absorption as the final structural model (Table 3). Since the simulated changes in FTC clearance (compared to pre-pregnancy) for each trimester were commensurate with the change when from pre-pregnancy to pregnancy (all trimesters combined), we used combined data from all trimesters in the final model. Pregnancy increased the apparent clearance by 63.1% compared to the non-pregnant baseline value, reducing the OFV by 27.685. As with TFV, we found a high proportional error of the Partners Demonstration Project data (85.4%). The diagnostic plot (Figure 3) showed some bias. The VPC (Figure 4) indicated the satisfactory performance of the final model. Our final model overestimated FTC plasma concentrations compared to those found in the Coleman et al. study.

Clinical trial simulation

In the non-pregnant population, the simulated median steady-state trough plasma concentration was 62.5 ng/ml for TFV and 158 ng/ml for FTC. Our simulation indicated that 13.9% and 16.4% of the participants on a standard “pre-pregnancy” regimen would have steady-state trough plasma TFV and FTC concentrations below the estimated protective threshold, respectively. In the standard TDF/FTC dosing arm (arm 1), the simulated median steady-state plasma TFV trough concentration dropped to 45.9 ng/ml, 39.3 ng/ml, and 27.3 ng/ml in the 1st, 2nd, and 3rd trimesters, respectively. According to our simulations, steady-state median TFV plasma concentrations decrease by 26.5–56.3% throughout pregnancy from a pre-pregnant baseline. Accordingly, we found that 34.0%, 43.8%, and 65.1% of steady-state plasma trough concentrations dropped below the estimated protective TFV trough concentration (35.5 ng/ml) due to the progressively increased clearance in the three trimesters. By comparison, in the simulated arm 2 pregnancy-adjusted double-dose group, the simulated median steady-state plasma trough concentration were 91.8 ng/ml, 78.7 ng/ml, and 54.6 ng/ml in the 1st, 2nd, and 3rd trimesters. Only 10.7%, 14.4%, and 27.8% of participants in the pregnancy-adjusted double-dose arm had



steady-state trough plasma concentrations less than 35.5 ng/ml (Figure 5).

For FTC, since all trimesters were combined in the final model, the simulated steady-state trough concentration estimates trough concentrations throughout pregnancy. In the arm 1 typical dosing group, the median simulated steady-state trough plasma concentration during the pregnant period was 62.4 ng/ml. During pregnancy, 42.1% of the steady-state trough concentrations dropped below the estimated protective trough concentrations for FTC (49.1 ng/ml). In the pregnancy-adjusted double-dose arm, the median simulated steady-state trough plasma concentration was 125 ng/ml; 22.4% of participants had trough concentrations less than 49.1 ng/ml, similar to the non-pregnancy group (Figure 6).

Discussion

We analyzed sparsely sampled PK data from the Partners Demonstration Project and intensively sampled PK data from the Coleman et al. study for both plasma TFV and FTC using a nonlinear modeling framework. Removal of outlier values had a modest impact on the popPK parameter estimates, but resulted in significantly improved model performance measures (pcVPC). The central volume of distribution estimate after removal of the outliers is consistent with that reported in the literature (15, 34).

We observed a progressive increase in clearance for TFV throughout pregnancy, with a nearly two-fold increase in clearance in the 3rd trimester compared to the non-pregnant baseline, and an associated progressive decrease in trough plasma levels. For

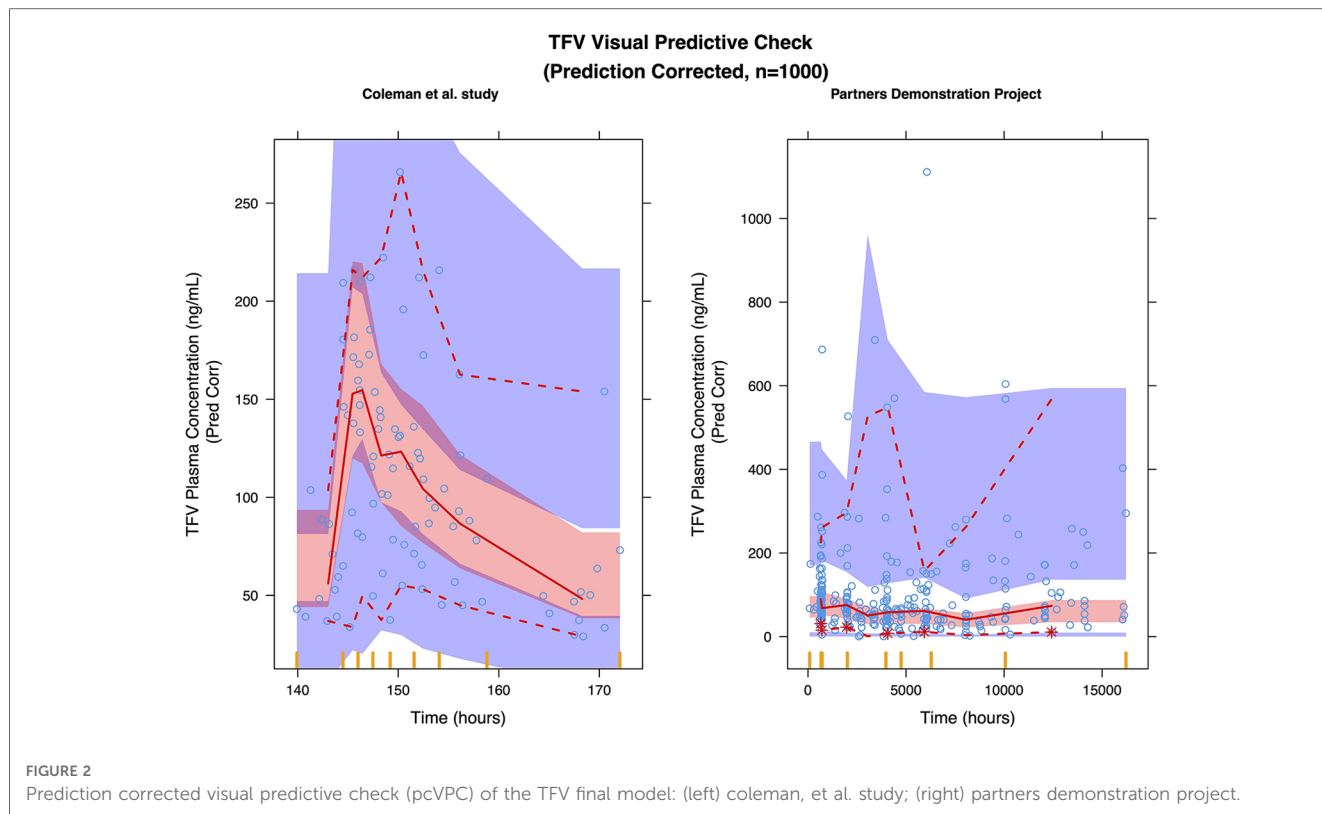


TABLE 3 Final estimates of FTC pharmacokinetic parameters, between subject variability, and residual variability^a.

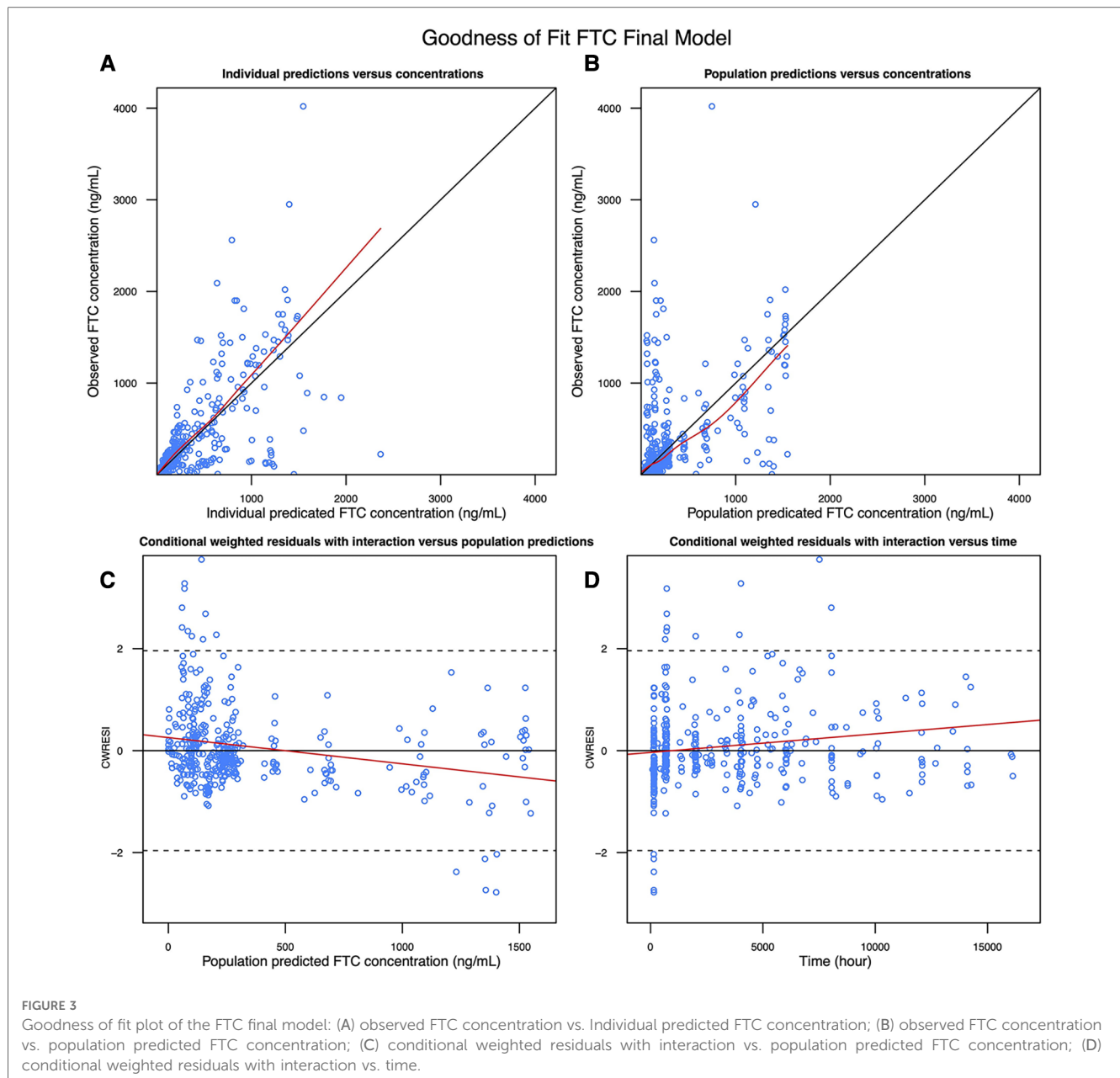
Parameter	Estimate	RSE%
CL/F (L/h)	16.7	9
V2/F (L)	58.8	62
Q/F (L/h)	13.8	22
V3/F (L/h)	190	18
KA (/h)	0.616	56
CL/F increment during pregnancy (%)	63.1%	23
BSV on CL/F	50.6%	9
BSV on Q/F	62.5%	43
BSV on V3/F	41.6%	60
BSV on KA	20.5%	26
σ1(prop; Coleman study)	29.3%	8
σ2(prop; partner demonstration project)	85.4%	6
σ2(add; partner demonstration project) (ng/ml)	12.5	31

^aCL/F, apparent clearance; V2/F, apparent volume of distribution of the central compartment; Q/F, apparent intercompartmental clearance; V3/F, apparent volume of distribution of the peripheral compartment; KA, absorption rate constant; BSV, between-subject variability; σ1, proportional residual error; σ2, additive residual error; RSE, relative standard error.

FTC, we observed a smaller increase in clearance in pregnancy, and an associated decrease in trough concentrations; these changes were consistent throughout pregnancy. Clinical trial simulation of standard vs. pregnancy-adjusted double-dose TDF/FTC regimens revealed that, compared to non-pregnant women, a clinically significant proportion of pregnant individuals on the standard dose would have exposures below the estimated protective thresholds for both TFV (35.5 ng/ml) and FTC (49.1 ng/ml) in

part or all, respectively, of pregnancy. In contrast, the pregnancy-adjusted dosing regimen significantly reduced the proportion of pregnant individuals falling below the estimated protective threshold from 34%, 43.8%, and 65.1%, to 10.7%, 14.4%, and 27.8% for TFV during the 1st, 2nd, and 3rd trimesters and from 42.1% to 22.4% for FTC during pregnancy. For context, the simulated 1st trimester steady-state plasma TFV trough concentrations with doubled TDF/FTC dosing—10.7% below and 89.3% above the 35.5 ng/ml daily dosing benchmark—is consistent with the 90% sensitivity threshold used in HPTN 066 to select the 35.5 ng/ml benchmark. Even so, the doubled TDF/FTC daily dose did not fully correct plasma TFV in the 2nd and 3rd trimester or FTC during pregnancy to pre-pregnant levels.

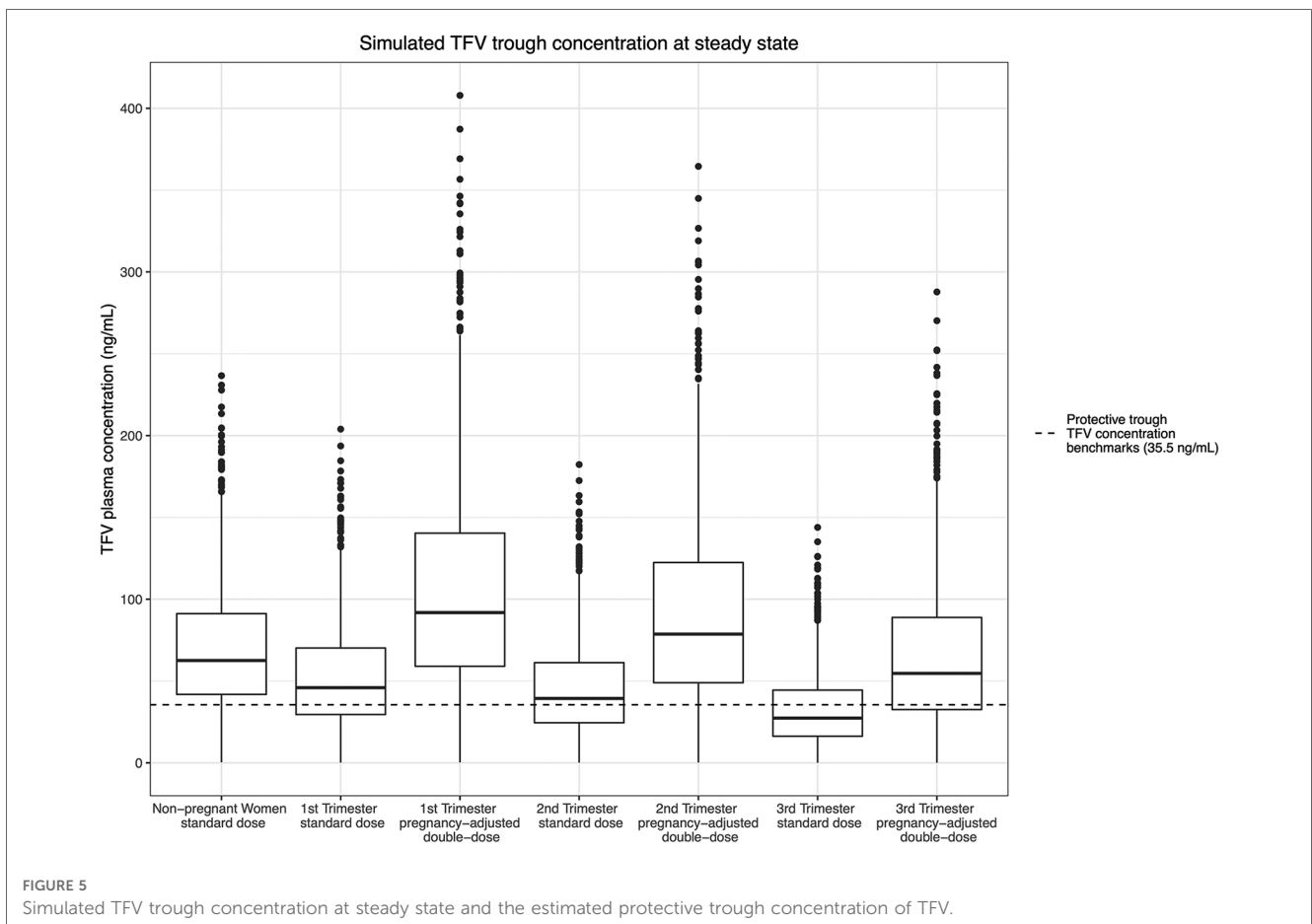
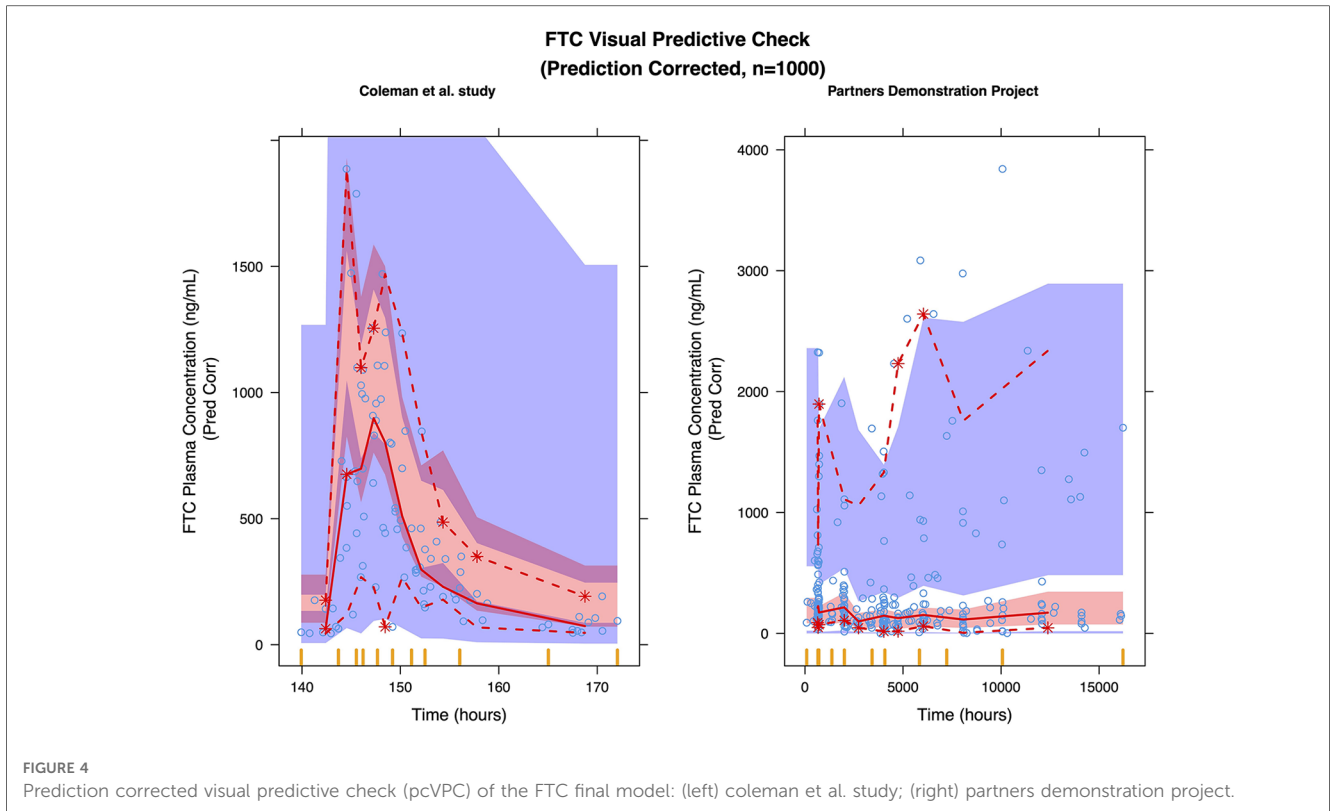
Consistent with our findings, physiological changes in renal blood flow are known to be progressive in pregnancy and are associated with progressive increases in clearance and decreases in exposure for renally excreted drugs, such as TFV and FTC. Although not identified in our final model, the increased volume of distribution during pregnancy may also contribute to lower plasma concentrations of TFV and FTC. The 26.5–56.3% reduction we estimated in simulated TFV trough plasma concentrations throughout the pregnancy is consistent with the 45%–58% reduction in TFV concentration reported by Pyra et al. in the averaged TFV concentrations in the Partners Demonstration Project (1). A popPK analysis by Benaboud et al. found a 39% increased clearance during pregnancy in women with HIV on TDF/FTC-containing regimens (2). A whole body physiologically based pharmacokinetic (PBPK) model by De Sousa Mendes et al. in pregnancy predicted a 40% increase in TFV apparent clearance

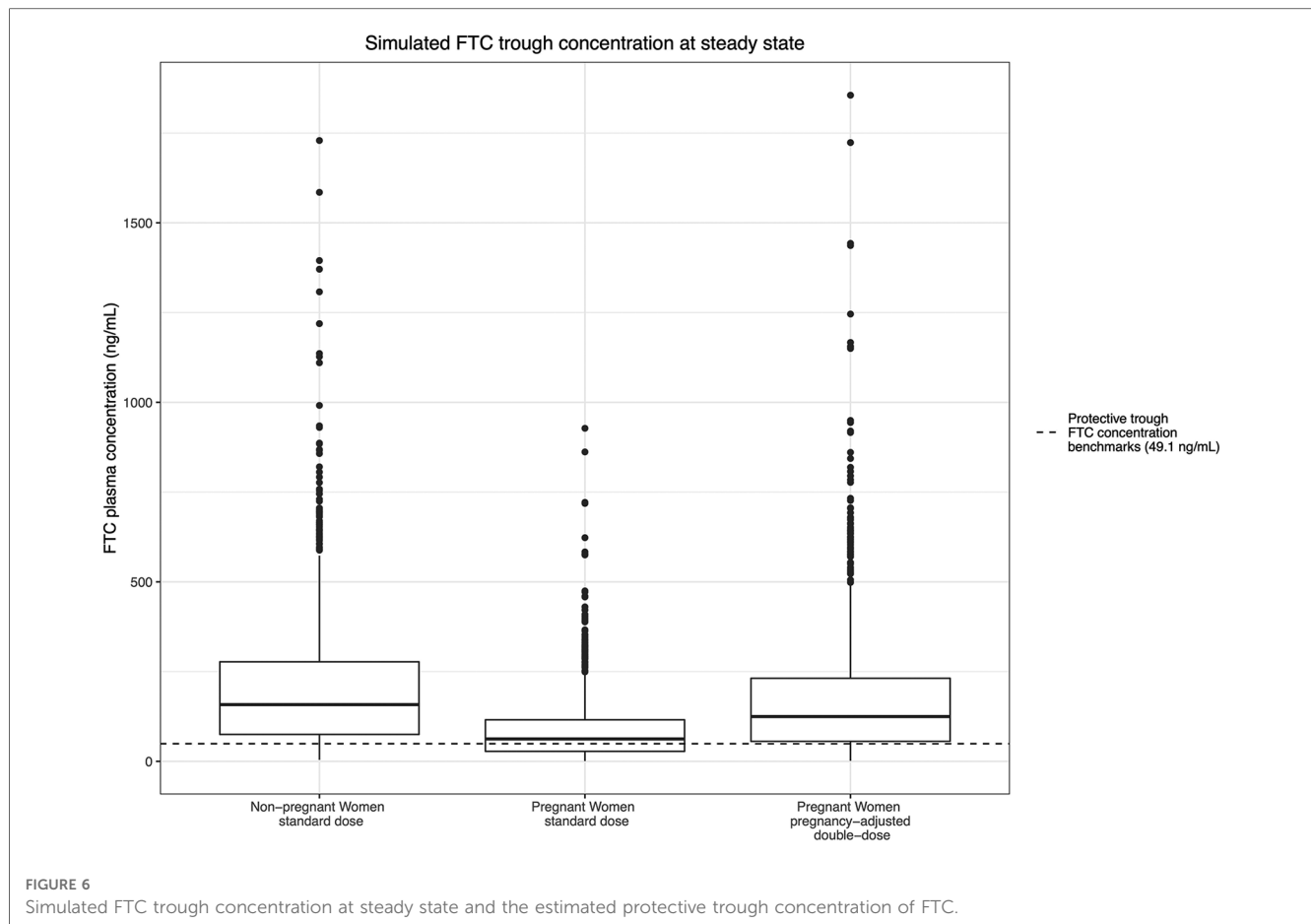


at approximately 33 weeks gestational age (35). A popPK model in women with HIV during pregnancy developed by Hirt et al. showed up to a 50% increase in the apparent clearance of FTC compared with the non-pregnant population (11), similar to our final estimates. A simplified pregnant-PBPK model developed by Xia et al. predicted a 1.39-fold change in the renal clearance of FTC in late pregnancy due to increased renal secretion and filtration (36). The whole body PBPK model mentioned above predicted a 1.29-fold clearance change, which is slightly lower than our estimates (35). Liu et al. predicted the PK profiles of FTC at different stages of pregnancy using a maternal-fetal PBPK model. They predicted an up to 27.7% decrease in median FTC AUC at 26 weeks of gestation (37).

Despite the availability of newer PrEP modalities, TDF/FTC remains the main stay of HIV prevention in pregnancy.

Although there are safety and PK data for HIV treatment in cisgender women, including during pregnancy, tenofovir alafenamide (TAF)/FTC is not yet recommended in cisgender women for PrEP given the lack of efficacy data. There are only limited safety and PK data for long-acting Cabotegravir in pregnancy (38, 39) and although there are reassuring safety data on the use of the Dapivirine ring in pregnancy (40, 41), its approval is limited globally. Decreased protective efficacy of TDF/FTC PrEP during pregnancy due to lower TFV and FTC exposures, as indicated in the clinical trial simulation, is cause for considerable concern, especially as the baseline HIV incidence among pregnant and postpartum women is two to four times that of non-pregnant women (42, 43). Modeled infectivity from the Partners in Prevention HSV/HIV Transmission Study and the Partners PrEP study demonstrated that the probability of





HIV acquisition per condomless sex act increases starting in early pregnancy and peaks postpartum [adjusted RR 3.97 (1.50, 10.51) $p < 0.001$] (43). Data from several observational studies corroborate that model's findings of increased male-to-female transmission in pregnancy (42–48). This increased incidence is attributed to both behavioral and biological changes (including immunological, vaginal microbiome, and vaginal epithelial integrity) during pregnancy and delivery (42, 46, 49–51). Prevention of HIV is especially critical in pregnant individuals secondary to the additional and increased risk of perinatal transmission. The risk of perinatal transmission is 9–15-fold higher in women diagnosed with HIV during (vs. prior to) pregnancy (22 vs. 1.8%) (52, 53). Increased HIV acquisition attributable to decreased protection of TDF/FTC PrEP against HIV during pregnancy has not been reported, but it is unclear if this is due to the adequacy of TDF/FTC PrEP protection in pregnancy vs. underutilization of PrEP in pregnancy and a dearth of large-scale research on PrEP in pregnancy. Limited clinical trials and epidemiologic research have focused on oral PrEP in pregnancy, but none in sufficient size to evaluate increased incidence due to TDF/FTC PrEP failure.

Limitations of the current analysis include the availability in pregnancy of only sparse PK data and only plasma drug concentrations, rather than active intracellular phosphorylated analytes. Additionally, we did not include body weight and renal

clearance as covariates or intracellular metabolite concentrations in our model. Neither study controlled for diet nor timing of dose related to meals, which could introduce additional variability (54, 55). Regarding the differences in CrCl between populations, kidney estimation equations were primarily derived in non-Black populations, and the equations used in the United States at the time the original data were collected (e.g., Coleman et al.) are not always applicable to African populations (e.g., Partners Demonstration Project). Previously published models found body weight (10) and creatinine clearance (10, 34, 56–58) to be significant covariates for TFV and FTC clearance. Even without inclusion of these covariates, the model still captures the global effect of trimester on clearance for TFV and underscores the need for a pooled analysis of all clinical trial data in pregnancy to better understand the dose optimization needs and for prospective PK research on dosing in pregnancy. For FTC, we were unable to identify the different changes in its clearance over different trimesters. An additional limitation was the need for a separate residual error model for Partners Demonstration Project; of particular concern was the large proportional error potentially attributable to differences in PK sampling and ascertainment of the dosing history. During our clinical trial simulation, we discovered that up to 65.1% of the TFV trough concentration and 45.2% of the FTC trough concentration in pregnant population may fall below the protective threshold.

However, we also observed that our final model tended to overestimate the TFV and FTC concentration in the non-pregnant population. As a result, the proportion of pregnant individuals with trough concentrations below the protective threshold (based on empiric observations) may have been underestimated. An additional limitation is that although Partners Demonstration Project utilized MEMS to measure adherence, doses were not observed and activation of the MEMS without taking a dose or taking a double dose (“catch up dosing”) prior to a study visit could bias CL/F. Lastly, as noted above, our sample size and that of published studies are insufficient to assess any impact of pregnancy on TDF/FTC PrEP efficacy.

Our popPK model and clinical trial simulation found that steady-state TFV and FTC trough plasma concentrations decreased during pregnancy, which puts pregnant individuals receiving standard TDF/FTC dosing at significantly greater risk of falling below the protective thresholds for both TFV and FTC compared to participants taking the pregnancy-adjusted double dose. This simulation provides the quantitative basis for the design of prospective TDF/FTC studies during pregnancy to evaluate the safety and appropriateness of pregnancy-adjusted dosing.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: Available upon request. Requests to access these datasets should be directed to chendrix@jhmi.edu.

Ethics statement

The studies involving humans were approved by Johns Hopkins University Institutional Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants’ legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

RS and CH conceived of the study. YY and RB developed the model and performed the in-silico simulation. RS and YY wrote the manuscript with support from CH and RB. All authors

discussed the results and contributed to the final manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

CH holds two patents related to HIV prevention and is founder of Prionde Biopharma, LLC, an HIV prevention product company. RB serves as a consultant for Advanced Bioscience Laboratories (NIAID). RS is the recipient of grant funding from Gilead Sciences, Inc. and ViV Healthcare, managed by MedStar Health. MM is the recipient of grant funding from Gilead Sciences, Inc. and Merck. CH is the recipient of grant funding from Gilead Sciences, Inc. and Merck.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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