

# UCSF

## UC San Francisco Previously Published Works

### Title

Characterizing HIV-Preventive, Plasma Tenofovir Concentrations-A Pooled Participant-level Data Analysis From Human Immunodeficiency Virus Preexposure Prophylaxis Clinical Trials.

### Permalink

<https://escholarship.org/uc/item/8tb4195s>

### Journal

Clinical Infectious Diseases, 75(11)

### ISSN

1058-4838

### Authors

Garcia-Cremades, Maria  
Vučićević, Katarina  
Hendrix, Craig W  
[et al.](#)

### Publication Date

2022-11-30

### DOI

10.1093/cid/ciac313

Peer reviewed

# Characterizing HIV-Preventive, Plasma Tenofovir Concentrations—A Pooled Participant-level Data Analysis From Human Immunodeficiency Virus Preexposure Prophylaxis Clinical Trials

Maria Garcia-Cremades,<sup>1,a</sup> Katarina Vučićević,<sup>1,2</sup> Craig W. Hendrix,<sup>3</sup> Priya Jayachandran,<sup>1</sup> Leah Jarlsberg,<sup>1</sup> Robert Grant,<sup>4</sup> Connie L. Celum,<sup>5</sup> Michael Martin,<sup>6,7</sup> Jared M. Baeten,<sup>5</sup> Jeanne Marrazzo,<sup>8</sup> Peter Anderson,<sup>9</sup> Kachit Choopanya,<sup>10</sup> Suphak Vanichseni,<sup>10</sup> David V. Glidden,<sup>11</sup> and Radojka M. Savic<sup>1</sup>

<sup>1</sup>Department of Bioengineering and Therapeutic Sciences, University of California San Francisco, San Francisco, California, USA; <sup>2</sup>Department of Pharmacokinetics and Clinical Pharmacy, University of Belgrade-Faculty of Pharmacy, Belgrade, Serbia; <sup>3</sup>Division of Clinical Pharmacology, Department of Medicine, Johns Hopkins University, Baltimore, Maryland, USA; <sup>4</sup>Department of Medicine, University of California San Francisco, San Francisco, California, USA; <sup>5</sup>Departments of Global Health, Medicine, and Epidemiology, University of Washington, Seattle, Washington, USA; <sup>6</sup>Centers for Disease Control and Prevention, National Center For HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Division of HIV/AIDS Prevention, Atlanta, Georgia, USA; <sup>7</sup>Thailand Ministry of Public Health-US CDC Collaboration, Nonthaburi, Thailand; <sup>8</sup>Division of Infectious Diseases, University of Alabama at Birmingham Medical Center, Birmingham, Alabama, USA; <sup>9</sup>Department of Pharmaceutical Sciences, University of Colorado, Denver, Colorado, USA; <sup>10</sup>Bangkok Tenofovir Study Group, Bangkok, Thailand; and <sup>11</sup>Department of Epidemiology and Biostatistics, University of California San Francisco, San Francisco, California, USA

**Background.** Daily dosing of tenofovir disoproxil fumarate, with or without emtricitabine, has high efficacy in preventing human immunodeficiency virus (HIV) infection when individuals are adherent. The target protective plasma concentration of tenofovir (TFV), however, is not fully understood. The aim of this study is to estimate the protective TFV plasma concentration.

**Methods.** Participant data from TFV-based daily oral and topical active arms of phase 3 trials (iPrEx, VOICE, and Partners PrEP) were pooled ( $n = 2950$ ). Individual specific risk scores (low and high risk) of acquiring HIV, based on an earlier placebo analysis, were created. Longitudinal TFV pharmacokinetics (PK), HIV outcome, individual risk scores and the effect of sex at birth data were integrated and analyzed using non-linear mixed effects models.

**Results.** Around 50% of the individuals were estimated to be adherent, which differed from self-reported adherence (~90%) and large variation between longitudinal adherence patterns were identified. Following oral administration, the estimated protective TFV trough concentration was substantially higher in high-risk females (45.8 ng/mL) compared with high-risk males (16.1 ng/mL) and to low-risk individuals (~7.5 ng/mL). Dosing simulations indicated that high-risk women require full adherence to maintain protective levels.

**Conclusions.** Using the largest PK-HIV outcome database to date, we developed a population adherence-PK-risk-outcome model. Our results indicate that high-risk females need higher levels of plasma TFV to achieve HIV protection compared with males. HIV protection exceeds 90% in all populations if daily adherence is achieved.

**Keywords.** HIV; preexposure prophylaxis; drug protective plasma concentration; HIV outcome.

Human immunodeficiency virus (HIV) infection continues to be a major global health issue with 1.5 million people becoming newly infected every year [1]. Preexposure prophylaxis (PrEP) using tenofovir (TFV), formulated as tenofovir disoproxil fumarate (TDF) with and without emtricitabine (FTC), has been evaluated in several randomized controlled trials in high-

risk populations [2–8]. PrEP efficacy results have varied among the trials; the highest efficacy results have been in heterosexual serodiscordant couples (75%) and high-risk heterosexual men and women (62%) compared with placebo [4, 5]. In 2 studies in young women at high sexual risk, PrEP failed to show a significant decrease in HIV infections compared with placebo [3, 8]. Poor PrEP adherence, as measured by low detectable drug levels, may explain this difference [9].

PrEP in these trials was dosed daily, which may have been a challenge for participants [3, 10–14]. Although daily dosing is recommended, less than daily dosing may still provide protection in some populations (eg, a 4-tablet, 3-dose on demand [2 + 1 + 1] regimen of TDF/FTC in males who have sex with males [MSM] [15]).

Several adherence questions remain unanswered: will an individual be protected from infection if he or she misses a single

Received 30 November 2021; editorial decision 10 April 2022; published online 26 April 2022

<sup>a</sup>Present address: Departamento de Farmacia Galénica y Tecnología Alimentaria, Facultad de Farmacia, Universidad Complutense de Madrid, 28040, Madrid, Spain.

Correspondence: R. M. Savic, Department of Bioengineering and Therapeutic Sciences, University of California San Francisco, San Francisco, 1700 4th St, Room 503-C, San Francisco, CA 94158 (rada.savic@ucsf.edu).

Clinical Infectious Diseases® 2022;75(11):1873–82

© The Author(s) 2022. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com

https://doi.org/10.1093/cid/ciac313

dose? How many doses can an individual miss before he or she is no longer protected, and how long before an exposure must PrEP be taken? Are both plasma and tissue drug concentrations needed, can protective drug levels be achieved with on-demand and/or long-acting formulations? Knowing the drug concentration that prevents HIV acquisition allows researchers to explore adherence patterns necessary to maintain a high level of protection from HIV infection.

We pooled longitudinal pharmacokinetic (PK) data and HIV outcomes from TFV-based treatment arms of 3 PrEP randomized controlled trials [2–4]. With this database, we used a novel quantitative approach to estimate the protective plasma TFV concentration. The proposed protective level may be used to distinguish HIV seroconversion because of nonadherence compared to suboptimal PK levels because of partial adherence and/or individual PK parameters. Additionally, the results may be used to suggest patient-centric management strategies to improve adherence patterns with daily dosing and to develop clinical trial simulation tools to propose novel PrEP regimens and study designs.

## METHODS

### Clinical Trials and Study Design

This analysis used individual participant-level data from pivotal TFV-based PrEP phase 3 RCTs: iPrEx [2] (NCT00458393), VOICE [3] (NCT00705679), and Partners PrEP [4] (NCT00557245). The trials were conducted in different target populations. iPrEx studied daily oral TDF-FTC compared with placebo in MSM and transgender women who have sex with men in Peru, Ecuador, Brazil, Thailand, South Africa, and the United States. VOICE evaluated daily oral TDF, oral TDF-FTC, oral placebo, 1% TFV vaginal gel, and placebo vaginal gel in young women at high sexual risk of HIV in South Africa, Uganda, and Zimbabwe. Partners PrEP compared daily oral TDF, oral TDF-FTC, and placebo in the HIV-negative partners of a HIV serodiscordant heterosexual couple in Kenya and Uganda. Analysis of the placebo arms was performed separately (M Garcia-Cremades, CW Hendrix, P Jayachandran et al, manuscript in preparation). Participants' characteristics, longitudinal PK, adherence, sexual risk behaviors, and HIV status data were available from the primary publications [2–4].

### Data Analysis and Model Building Process

Longitudinal PK and HIV outcome data were linked using the population approach as implemented in Nonlinear Mixed Effects Models v7.4 [16]. Using this approach, data from the different studies were pooled to establish a concentration-prevention relationship and identify the TFV concentration associated with a 90% decrease in the probability of HIV infection in different risk subpopulations. Individual probability of being adherent to daily PrEP, estimated from individual PK data, was also considered. Through model simulations, adherence patterns required to

achieve and maintain protective concentrations can be identified to inform clinical practice and research. This analysis was performed sequentially. First, the adherence-PK models for both oral and vaginal TFV administration were developed. Interindividual variability was modelled exponentially and residual error was described using a combined error model. Second, a derived PK metric (eg, average or trough concentration) was linked to HIV outcome. Finally, the impact of PrEP in different risk groups following different adherence patterns was evaluated through model simulations using the PKPDsim R package. Data management, model selection, and evaluation are described in [Supplementary Methods](#).

### Adherence-PK Model

The TFV PK profile was described using a 2-compartment model. The structural PK model initially characterized the oral administration data; subsequently, vaginal gel administration data were included. Participants' sex, race, body weight, age, and creatinine clearance (CrCl) were tested as covariates. Below limit of quantification (BLQ) observations were handled by estimating the likelihood of the predicted TFV concentration being less than the lower limit of quantification [17].

To address nonadherence or low adherence suspected in the studies, a mixture modeling approach was used on the apparent drug bioavailability ( $F$ ) [18]. A mixture model is a probabilistic model for identifying the presence of subpopulations, without a requirement that this information be present in the observed data (eg, belonging to an adherent ( $F = 1$ )/nonadherent ( $F < 0.001$ ) subpopulation). Longitudinal visit-specific probabilities of drug intake were estimated and combined with individual PK parameters to reconstruct average PK profiles for each follow-up visit using the following equation:

$$C_{avg} = \frac{Dose \times F \times p_{adherence}}{CL \times \tau}$$

$C_{avg}$  represents the average TFV concentration,  $CL$  the individual predicted clearance,  $p_{adherence}$  the probability of drug intake for each visit, and  $\tau$  the dosing interval.

### HIV Outcome Model

The primary endpoint in the studies was HIV seroconversion of initially seronegative individuals. HIV outcome was tested monthly. Time to seroconversion was calculated based on enrollment and seroconversion date and modeled using a parametric survival function [19, 20].

After developing the baseline survival model, calculated individual  $C_{avg}$  was evaluated as a driver of HIV protection:  $C_{avg}$  closest to the time of infection (HIV-positive individuals) and median  $C_{avg}$  over study period (HIV-negative individuals). The target  $C_{avg}$  concentrations associated with 90% decrease in the probability of HIV ( $EC_{90}$ ) were transformed to target trough concentrations ( $C_{trough}$ ) using PK simulations

(Supplementary Methods). The effect of sex at birth and treatment arm were evaluated as covariates.

Finally, the impact of an individual's underlying risk of infection on the ability to define an accurate PK-HIV outcome relationship was evaluated. The probability of HIV infection over time for each participant was simulated using the parametric HIV risk models developed from the individual risk factors of the placebo arms (M Garcia-Cremades, CW Hendrix, P Jayachandran et al, manuscript in preparation). HIV risk level was stratified based on these simulations to identify a moderate- to high-risk and low-risk subpopulation, which were individuals with an infection probability higher or lower than the overall median probability (7.7%) at week 150 in the pooled dataset, respectively. Different pharmacodynamic (PD) parameters were estimated for the different subpopulations. Figure 1 shows the schematic representation of the PKPD model.

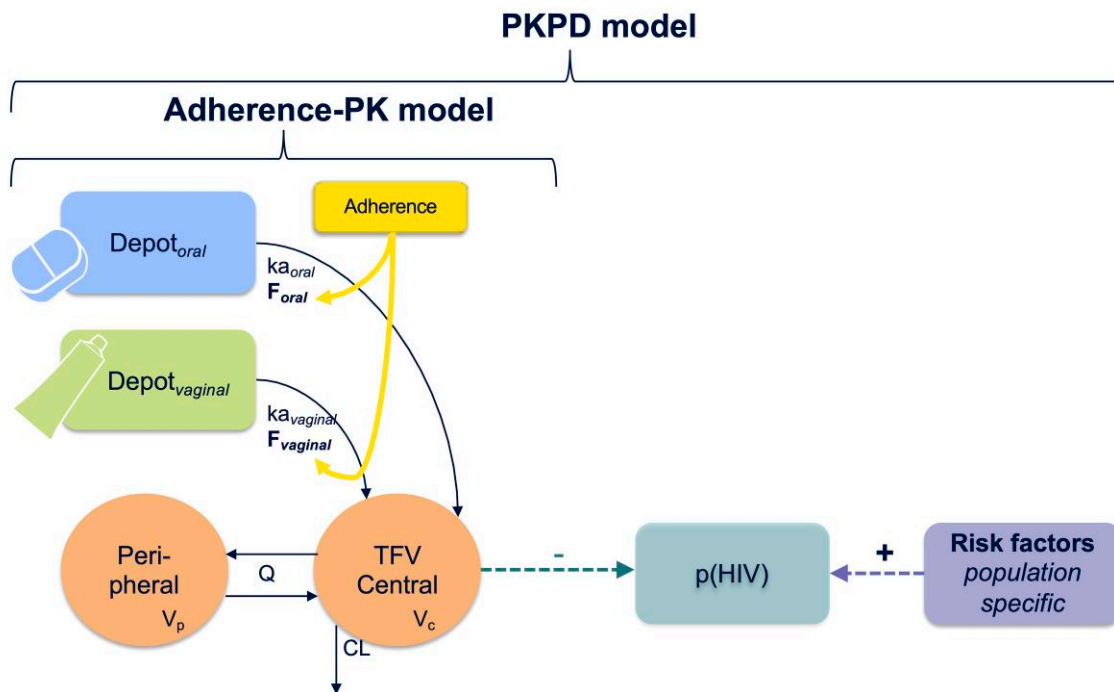
## RESULTS

### Largest Database of HIV Outcome and Tenofovir PK Levels From Different High-risk Populations

Of 7440 participants from the active arms of iPrEx, Partners PrEP, and VOICE, 3213 (43.2%) provided at least 1 PK measurement, and 2950 (39.7%) were included in the analysis

(Figure 2). The final dataset contained 243 individuals who seroconverted. Baseline demographics and PK data characteristics for the analysis dataset and the total study randomized participants are listed in Table 1 and Supplementary Table 1, respectively. The final pooled dataset constitutes, to the best of our knowledge, the largest database including both longitudinal HIV outcome and PK data profiles.

High variability in the TFV plasma levels was observed (Supplementary Figure 1A). Approximately 43% (oral) and 76% (vaginal gel) of the measured concentrations were BLQ and were almost uniformly distributed throughout the 100-hour postdose period. The BLQ distribution among trials was considerably different (Table 1), with the highest proportion of BLQs (75%) in the vaginal arm of the VOICE trial, which may partly explain the high proportion of HIV seroconversions (11%) by the end of follow-up (Supplementary Figure 1C). HIV seroconversions occurred regardless of PK level at some visits (Supplementary Figure 1B); however, individuals with BLQ values closest to time of infection or time of censor had a greater probability of HIV infection, followed by those with low PK levels (below the 33rd percentile) (Supplementary Figure 1D). Figure 3 explores the proportion of HIV-positive individuals

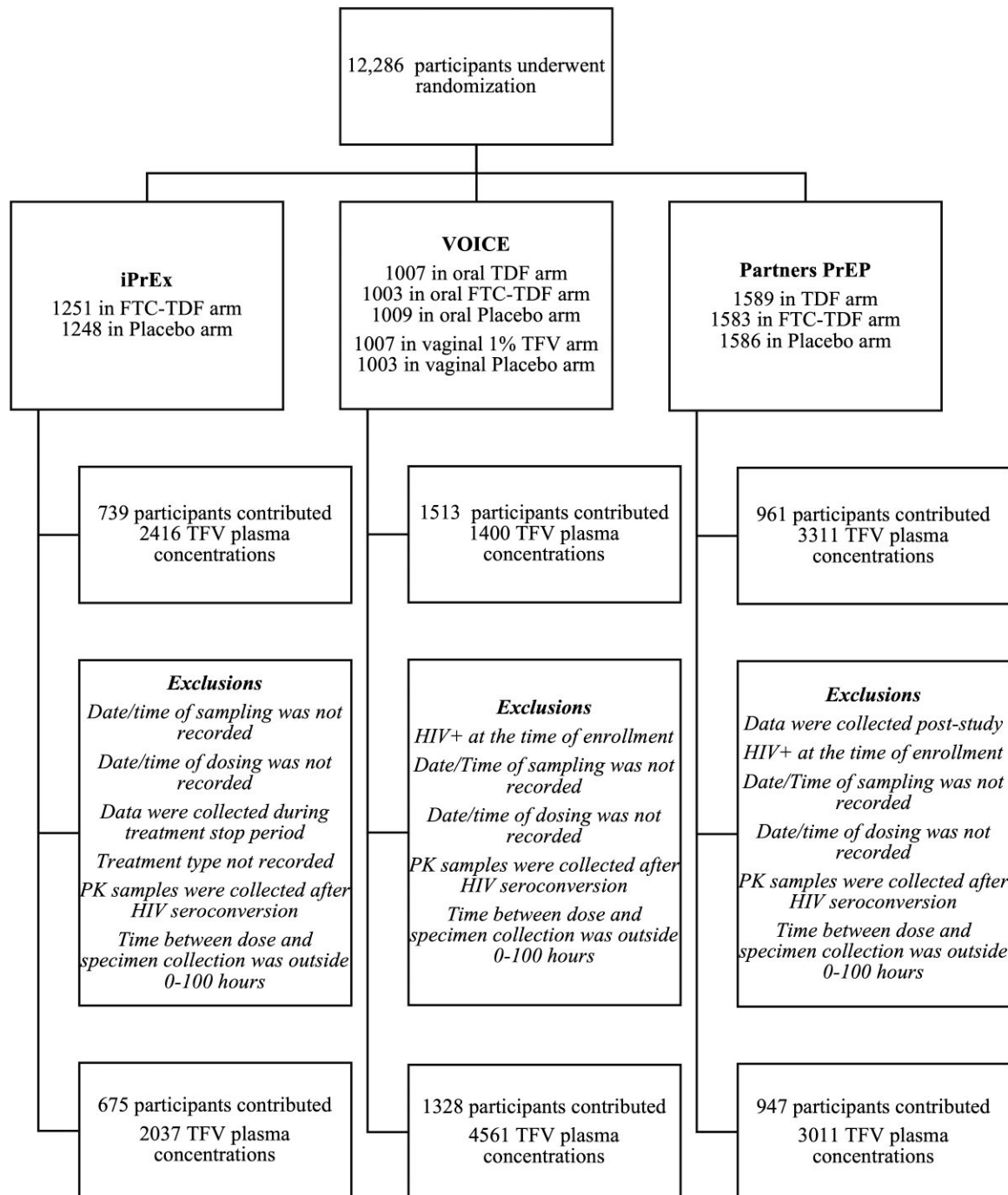


**Figure 1.** Schematic representation of the PKPD model. The full PKPD model links the adherence-PK model for both routes of administration to the probability of acquiring HIV infection over time. The population-specific risk factors refer to the predictors of HIV risk for each study that were identified in the companion submitted paper.  $p(\text{HIV})$  represents the probability of HIV. PK parameter definition:  $k_a$ , absorption rate constant. CL, clearance; F, drug bioavailability; HIV, human immunodeficiency virus; Q, inter-compartmental clearance;  $V_c$  and  $V_p$ , volume of central and peripheral compartment, respectively.

in each group strata (HIV risk subpopulation and sex at birth) for oral administration arms. The highest proportion of HIV infections occurred in moderate- to high-risk women, and no statistical difference was found between iPrEX and Partners PrEP men when stratified by risk. Difference in protective levels between risk groups can be anticipated based on the distribution of their predicted  $C_{avg}$ .

#### Differentiation Between Nonadherence and Low PK: Estimated Individual Probability of Adherence

The population was split into 2 subpopulations: (1) adherent ( $F = 100\%$  for oral PrEP and 2.25% for vaginal PrEP) and (2) nonadherent ( $F \leq 0.001\%$  for both oral and vaginal PrEP). The overall probability of PrEP use was similar between oral and vaginal administration (55.4% and 49.7%, respectively),



**Figure 2.** Enrollment, follow-up, and pharmacokinetics (PK) inclusion criteria for study participants. FTC, emtricitabine; HIV, human immunodeficiency virus; PrEP, pre-exposure prophylaxis; TDF, tenofovir disoproxil fumarate; TFV, tenofovir.

with large between and within (visit-to-visit) individual variability. This estimated adherence of ~50% is lower than the self-reported adherence of nearly 90% reported by research participants during the clinical trials. Individual adherence patterns appear to change over time and different patterns of adherence and timing of seroconversion are shown (Supplementary Figure 2). One-hundred and twenty seven of 243 participants who seroconverted were fully nonadherent, 70 had mixed behaviors, and 46 appeared to be adherent.

The apparent CL (CL/F) increased by 2.3 L/h (4.1%) for each 10 mL/min increase in CrCl and 3.2 L/h (5.6%) for each 10 kg increase in weight. Females had a 44% lower apparent volume of distribution than males. Overall, male sex, normal CrCl, and normal body weight were all independently associated with lower plasma TFV concentrations when compared with women, abnormal creatinine clearance, and low body weight, respectively. Supplementary Table 2 and Supplementary

Figure 3 display the PK parameter estimates and PK model evaluation results, respectively.

#### Plasma TFV Levels and the Presence of FTC Are Associated With a Decrease of HIV Infection

Hazard of acquiring HIV infection, described by a surge distribution, was significantly associated with plasma TFV  $C_{avg}$  ( $P < .001$ ). The PD relationship was best described by a maximum effect model, estimating the concentration associated with 50% of the maximum effect ( $EC_{50}$ ) (Supplementary Table 3). Administration of oral TDF increased the baseline hazard by 70% compared with TDF/FTC.

#### The Underlying Individual HIV Hazard Is a Determining Factor in the Estimation of Accurate Preventive Concentrations

The inclusion of patient stratification based on underlying risk of infection in the PKPD model led to the estimation of significantly different  $EC_{50}$  values between risk groups for both oral and vaginal PrEP use ( $P < .001$ ) (Supplementary Table 3).

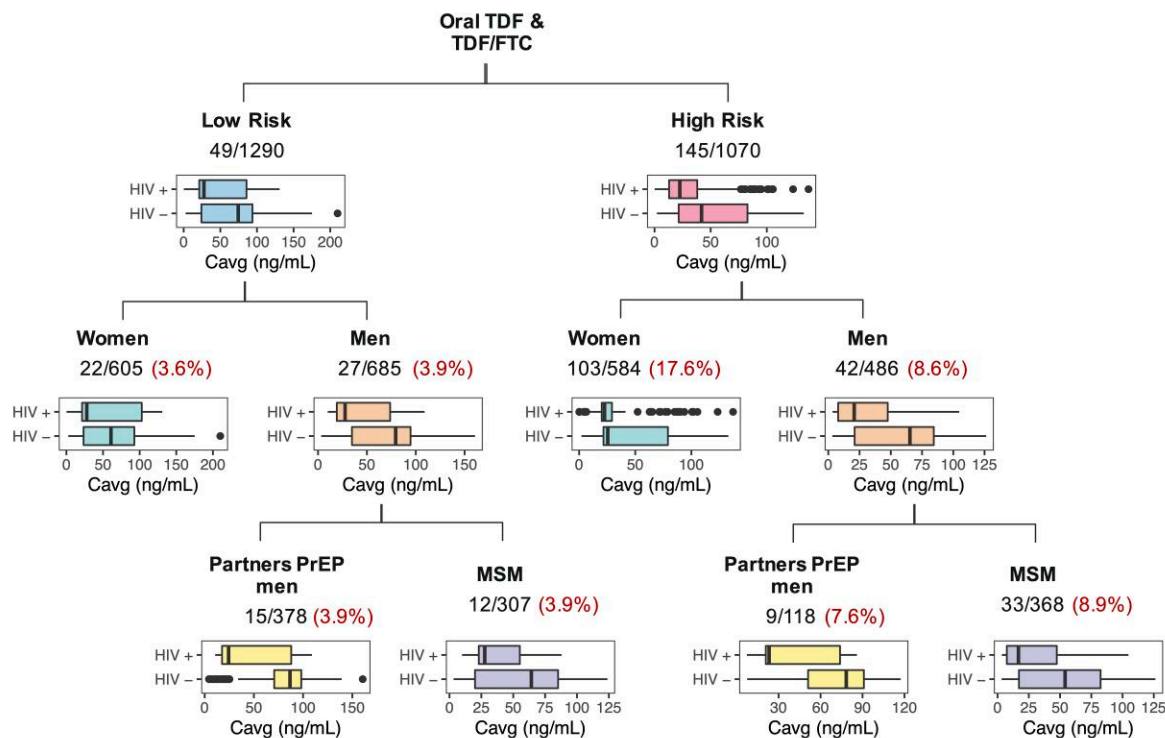
**Table 1. Baseline Characteristics and Pharmacokinetic Data From Participants in 3 Randomized Placebo-controlled HIV Preexposure Prophylaxis Trials**

Characteristics (Units)	Pooled Data n (%)	IPrEx n (%)	VOICE n (%)	Partners PrEP n (%)
<b>Participant's baseline characteristics</b>				
Number of individuals with valid PK data	2950	675	1328	947
<b>Number of individuals with valid PK data by treatment arm</b>				
TDF oral	790	0	351	439
FTC/TDF oral	1570	675	387	508
1% tenofovir vaginal gel	590	0	590	0
Sex at birth, male	1171 (39.7)	675 (100)	0	496 (52.4)
Age, median (range), y	27 (18–64)	25 (18–63)	25 (18–40)	32 (18–64)
Weight, median (range), kg	63 (38–153.81)	66 (44–153.81)	65 (38–138)	59 (39–118)
Creatinine clearance, median (range), mL/min	119 (50–140)	116 (63.5–140)	130 (61–130)	105 (50–140)
Acquired HIV infection during the study period	243 (8.2)	45 (6.7)	146 (10.9)	52 (5.5)
<b>PK data</b>				
<b>Number of PK level (&gt;BLQ) samples by treatment arm</b>				
Oral administration	4477	1077	922	2478
Vaginal gel administration	417	0	417	0
<b>Number of BLQ samples by treatment arm</b>				
Oral administration	3412	960	1919	533
Vaginal gel administration	1303	0	1303	0
<b>Median follow-up visits with PK data per individual, median (range)</b>				
Oral administration	3 (1–16)	2 (1–16)	3 (1–12)	3 (1–10)
Vaginal gel administration	2 (1–10)	0	2 (1–10)	0
<b>TFV level including BLQs, median (range), ng/mL</b>				
Oral administration	30.9 (0.25–899)	23.20 (10–899)	0.31 (0.25–466)	69.30 (0.31–812)
Vaginal gel administration	0.31 (0.25–40.9)	0	0.31 (0.25–40.9)	0
<b>TFV level excluding BLQs, median (range), ng/mL</b>				
Oral administration	78.1 (0.25–899)	94.7 (10.1–899)	61.4 (0.25–466)	79.2 (0.313–812)
Vaginal gel administration	1.14 (0.25–40.9)	0	1.14 (0.25–40.9)	0
<b>Risk stratification</b>				
Low risk	1480	307	532	641
Moderate–high risk	1470	368	796	306

PK values that were collected as not BLQ and were < BLQ (eg, 0.25 ng/mL in VOICE study) were kept as PK levels.

Abbreviations: BLQ, below limit of quantification (10 ng/mL iPrEx, 0.3 ng/mL VOICE, and Partners PrEP); FTC, emtricitabine; HIV, human immunodeficiency virus; PK, pharmacodynamics; TDF, tenofovir disoproxil fumarate; TVF, tenofovir.





**Figure 3.** Summary of HIV-positive and HIV-negative individuals and their  $C_{avg}$  levels distribution, for the different risk and sex at birth stratification groups for those in the oral administration arm (with or without FTC). Numbers indicate number of HIV seroconversions/total number of individuals in that subgroup. Proportion of HIV seroconversion by strata is shown in red. FTC, emtricitabine; HIV, human immunodeficiency virus; MSM, males who have sex with males; PrEP, preexposure prophylaxis; TDF, tenofovir disoproxil fumarate; TFV, tenofovir.

The distribution of this individual underlying risk of HIV infection is shown in Figure 4A. Fifty-four HIV infections occurred in the low-risk subgroup ( $n = 1480$ ) compared with 189 in the moderate- to high-risk group ( $n = 1470$ ). Figure 4 shows that the model adequately describes the different HIV risk profiles between risk groups (B) and when stratified by  $C_{avg}$  for the moderate- to high-risk individuals (C), especially for those who received oral PrEP.

#### Women Taking Oral PrEP Need Higher Plasma TFV Levels to Be Protective Compared With Men

The estimation of a different  $EC_{50}$  for men and women in the moderate- to high-risk subpopulation following oral administration ( $P < .05$ ) suggests that women need a higher protective TFV concentration than men (Supplementary Table 3). The target protective  $C_{trough}$  levels for moderate- to high-high risk was 16.1 ng/mL (men) and 45.8 ng/mL (women) following oral administration. Target protective concentrations in the low-risk subpopulation did not differ by sex at birth. As a note, regardless of selection of the cut-off between low and moderate- to high-risk subpopulation, women at higher risk show higher estimated target concentration compared with men at high risk (Supplementary Figure 4).

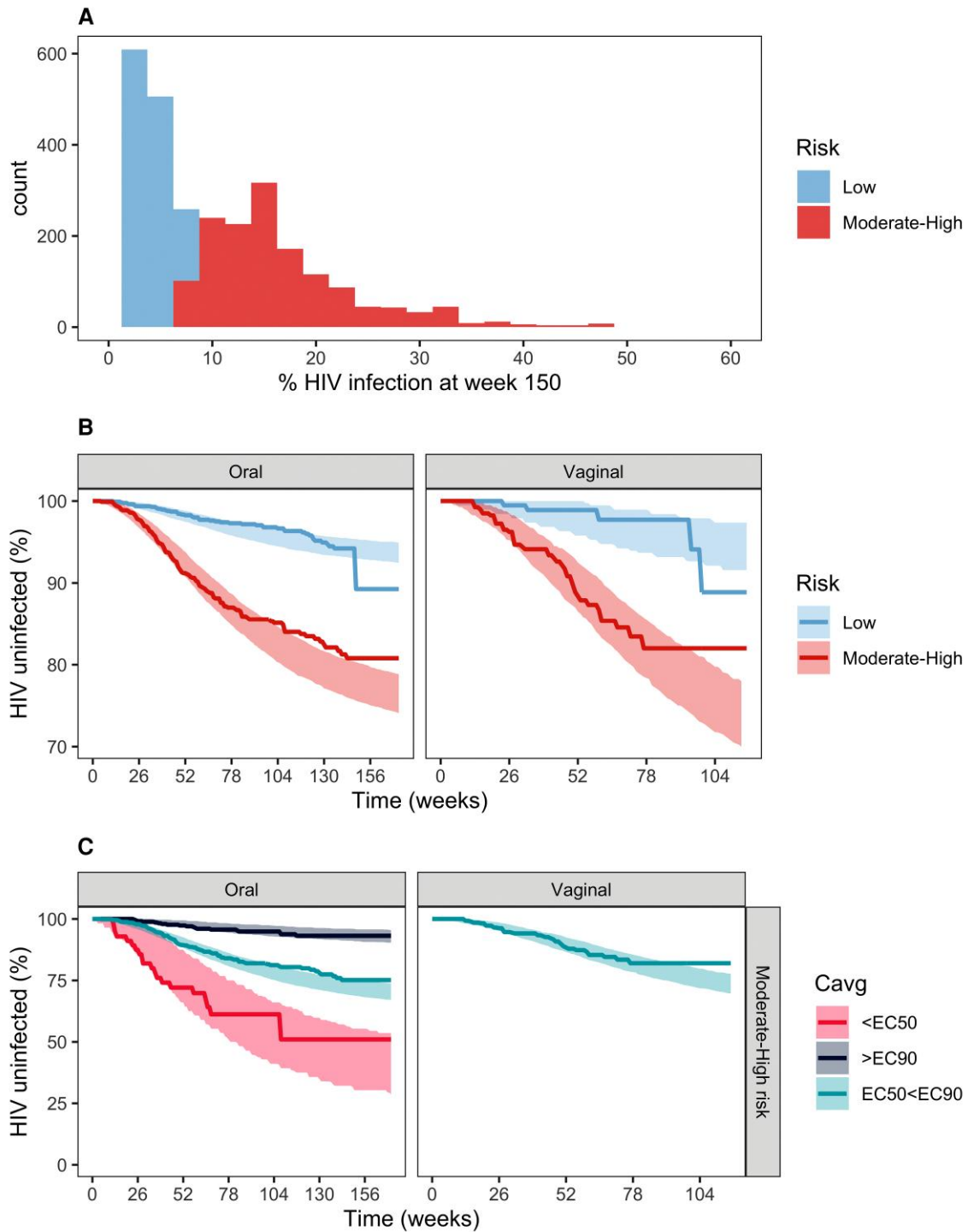
Figure 5 compares typical PK profile simulations following TDF oral administration (300 mg) for sample adherence patterns with the identified target concentrations for the different risk groups. Steady-state PK levels drop significantly below protective  $C_{trough}$  for those at high risk when the adherence pattern is reduced from daily to every 2 days or every 3 days, especially in women. Trough, average, and maximum concentrations related to the estimated  $EC_{50}$  and  $EC_{90}$  parameters are reported in Table 2.

#### Different Plasma Levels of Tenofovir Produce the Same Effect

The estimated  $EC_{50}$  parameters for both risk level groups were significantly different between oral and topical vaginal administration in women (Supplementary Table 3). These results reveal that different TFV plasma levels, differing by a factor of 2 on a log10 scale, achieved with different formulations and routes of administration, can produce the same protective effect. Target plasma concentrations following vaginal administration were estimated with less accuracy than following oral administration.

#### DISCUSSION

We performed an individual-level data pooled analysis to develop a population exposure-outcome model that describes

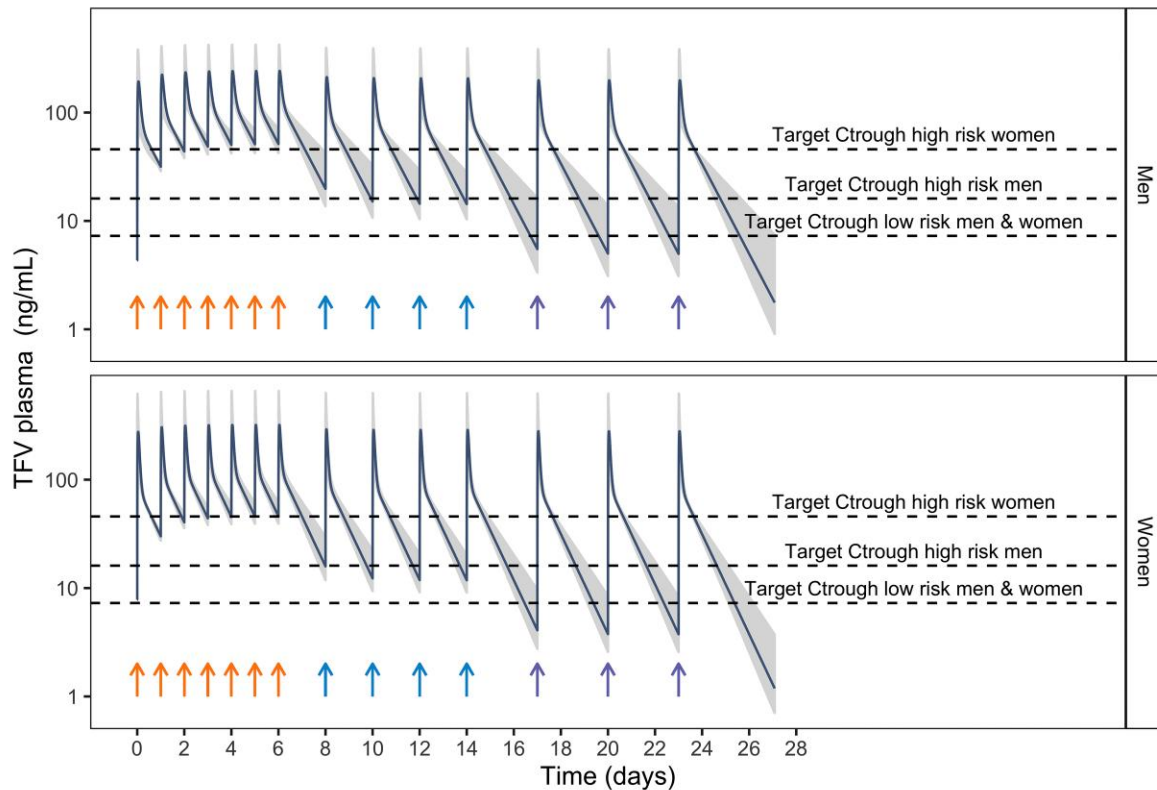


**Figure 4.** Final PKPD exploration and evaluation. (A) Predicted baseline HIV probability distribution of the pooled data using the risk predictor algorithm developed in the placebo arm analysis. (B) Kaplan-Meier VPC plot stratified by risk levels. (C) Kaplan-Meier VPC plot stratified by target concentration for moderate- to high-risk individuals. Continuous lines in the VPC plots represent real data; shaded areas represent 95% confidence intervals for simulated data. Moderate- to high-risk EC<sub>50</sub> and EC<sub>90</sub> values (C<sub>avg</sub>) are 3.53 and 31.7 ng/mL for men and 11.2 and 100.8 ng/mL for women, respectively. HIV, human immunodeficiency virus; PKPD, pharmacokinetics/pharmacodynamic.

TFV plasma concentrations and the probability of HIV seroconversion over time for various HIV infection risk levels. We proposed protective TFV concentrations (C<sub>trough</sub> 45.8 ng/

mL in women and 16.1 ng/mL in men at high risk, following oral administration), while also integrating the probability of being adherent to daily PrEP and the underlying individual





**Figure 5.** PK model simulations in men and women following oral administration of TDF (300 mg) for sample adherence patterns. Dark blue line represents the typical PK population profile, whereas gray shadows represent individual PK profiles (200) including interindividual variability. Dashed lines indicate the target  $C_{trough}$  levels associated with a 90% decrease in the probability of HIV for men and women at high-moderate subgroups and for those in a low-risk subgroup. Arrows indicate the time the dose is administered. Sample adherence patterns are daily (orange arrows), every 2 days (blue arrows), and every 3 days (purple arrows). HIV, human immunodeficiency virus; PK, pharmacokinetics; TDF, tenofovir disoproxil fumarate.

HIV infection risk as determined by the population-specific risk factors that we previously identified (M Garcia-Cremades, CW Hendrix, P Jayachandran et al, manuscript in preparation).

Our pooled dataset comprised 2950 individuals with valid TFV PK levels and 243 HIV infections. This dataset is larger than the largest clinical trial in our group (VOICE [3]). For example, other analysis that estimated protective concentrations included 200 participants and 30 HIV seroconversions [21]. Integrating data across several clinical trials enables us to use population modeling approaches with high predictive power. Additionally, our work promotes data sharing to encourage international collaboration in research to expand the impact of clinical trials [22].

Published results emphasize that nonadherence or low adherence to TDF-based PrEP, defined as nonquantifiable TFV plasma concentrations, is prevalent in participants with unfavorable HIV outcomes [2, 4–6]. In this analysis, we defined adherence as a probability of drug intake and estimated it for every visit and every subject. The visit specific ~50% probability of drug intake indicates that participants consumed half of

prescribed pills in the period preceding the visit. A mix of estimated adherence patterns was identified (eg, participants with high PK levels at the HIV seroconversion visit, but mostly non-adherent in previous visits). Hence, HIV seroconversion might be due to suboptimal plasma levels at the time of HIV exposure.

The use of oral TDF alone compared to oral TDF-FTC shows a 70% of increase of the baseline HIV hazard in this analysis. Animal studies suggest that the combination of TDF-FTC might provide higher protection from sexual HIV acquisition [23].

Our analysis showed that moderate- to high-risk female participants using oral PrEP need higher TFV levels in plasma than males. Likewise, both high-risk populations need greater adherence to maintain efficacy levels, and women more than men (Figure 5), which might be explained by tissue pharmacology [24]. Nevertheless, HIV protection exceeds 90% in all populations with daily adherence. A similar plasma  $EC_{90}$  ( $C_{avg}$  of 105–110 ng/mL) has been reported from a meta-analysis of PrEP trials, where adherence to daily dosing (roughly 7 doses per week), was also recommended [25]. Of note, emerging data from demonstration

**Table 2. Typical PK Metrics ( $C_{\text{trough}}$  and  $C_{\text{max}}$ ) Associated With the Estimated  $EC_{50}$  and  $EC_{90}$   $C_{\text{avg}}$** 

	$EC_{50}$ (ng/mL)			$EC_{90}$ (ng/mL)		
	$C_{\text{avg}}$	$C_{\text{max}}$	$C_{\text{trough}}$	$C_{\text{avg}}$	$C_{\text{max}}$	$C_{\text{trough}}$
PKPD model						
Oral administration <sup>a</sup>	2.73	8.68	1.24	24.57	78.12	11.15
Vaginal administration	0.0348	0.09	0.02	0.3132	0.83	0.15
PKPD model with risk stratification						
Oral administration <sup>a</sup>						
Low risk	2.48	7.84	1.26	22.32	70.96	10.13
Moderate–high risk	10.2	32.43	4.63	91.8	291.87	41.67
Vaginal administration						
Low risk	0.0179	0.05	0.01	0.161	0.43	0.07
Moderate–high risk	0.141	0.38	0.07	1.269	3.38	0.59
PKPD model with risk and sex at birth stratification (final model)						
Oral administration						
Low-risk men	1.78	4.27	0.9	16.02	38.4	8.15
Low-risk women	1.78	5.65	0.8	16.02	50.9	7.28
Moderate- to high-risk men	3.53	8.5	1.8	31.7	76.1	16.1
Moderate- to high-risk women	11.2	35.6	5.1	100.8	320.5	45.8
Vaginal administration						
Low risk	0.0179	0.05	0.01	0.161	0.43	0.07
Moderate–high risk	0.141	0.38	0.07	1.269	3.38	0.59

Abbreviations:  $C_{\text{avg}}$ , average concentration;  $C_{\text{max}}$ , maximum concentration;  $C_{\text{trough}}$ , trough concentration;  $EC_{50}$ , 50% of the maximum effect;  $EC_{90}$ , 90% of the maximum effect; PKPD, pharmacokinetics/pharmacodynamics.

<sup>a</sup>For the PKPD model and PKPD model with risk stratification, the PK simulations for calculating  $C_{\text{max}}$  and  $C_{\text{trough}}$  were performed in women.

projects are finding low rates of HIV infection in women with moderate adherence, supporting the efficacy of oral PrEP when adhered to [26]. Those results suggest that women at risk may achieve high enough adherence around HIV exposure times to provide high levels of protection. This analysis, however, could not identify differences in plasma TFV protection levels between males and females in the low-risk subpopulation, possibly because of the low number of HIV cases in that risk group.

Vaginal data obtained from the VOICE study were associated with a low adherence and a high number of plasma BLQ samples, which may have affected the estimation of accurate protective TFV levels in plasma following vaginal administration in moderate- to high-risk populations. TFV concentration after vaginal dose can also be substantially affected by medication adherence and the vaginal microbiome [27]. Nevertheless, different plasma levels of TFV, given different routes of administration, produced the same level of protection in this analysis, suggesting that TFV concentrations at the site of action should also be considered as a predictor of HIV infection. In fact, several studies have shown the potential of TFV measured in several biologic matrices (eg, peripheral blood mononuclear cells, vaginal/rectal tissue) as predictors of PrEP efficacy [28, 29]. As an example, daily oral dosing of tenofovir alafenamide (which delivers the active metabolite to target cells more efficiently, with lower plasma TFV concentrations) with FTC, has been demonstrated to be noninferior to daily oral TDF-FTC in MSM and transgender women [30]. Therefore, we are

performing additional analyses incorporating the target metabolite and its distribution to peripheral blood mononuclear cells in the current PKPD model to assess the predictive capability of TFV-DP in comparison to TFV. Understanding if the key predictor of HIV prevention is drug levels in plasma or in target cells would inform the selection on TDF or on tenofovir alafenamide-based oral formulations.

Results from this analysis provide target drug concentrations to inform the development of novel TFV-based PrEP formulations, which might help to overcome the adherence challenges associated with daily administration. Furthermore, these models can be used as a tool for clinical trial simulations to inform optimal study designs of these forthcoming TFV formulations.

A limitation of this analysis is the high number of BLQ samples, especially in the vaginal data obtained from VOICE study, which made it difficult the estimate of accurate target concentrations in the vaginal tissue. Additionally, the target protective concentrations for the different risk strata were estimated with low precision (Supplementary Table 3), hindering the reporting of protective  $C_{\text{trough}}$  confidence intervals for moderate- to high-risk men and women taking oral PrEP.

In conclusion, this analysis used the largest database to date of TFV placebo, PK plasma levels, and HIV outcome information. We found that individuals with different underlying HIV risk hazards may need different plasma protective levels, and that moderate- to high-risk women need higher plasma TFV levels to prevent HIV acquisition compared with men.

## Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

## Notes

**Acknowledgments.** The authors appreciate the contribution of the participants in the studies and the staff that worked on the trials.

**Disclaimer.** The findings and conclusions in this paper are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

**Financial support.** This work was supported by PhRMA Foundation (Postdoctoral Fellowship in Translational Medicine and Therapeutics to MG-C), and by the Bill and Melinda Gates Foundation (grant OPP1099837) (subaward from Johns Hopkins University to University of California San Francisco). C. W. H. reports support for the present work from the Bill and Melinda Gates Foundation: secondary data analysis contract, paid to Hopkins, managed by Hopkins. K. V. reports support for the present work: Fulbright scholarship under the Fulbright U.S. Scholar Program for stay and research at University of California San Francisco, during 2016–2017. P. A. reports support for this work from the National Institutes of Health (NIH) (paid to institution) and Gilead Sciences (donation of study drug).

**Potential conflicts of interest.** D. V. G. has served on an Advisory Board for Merck and has personal fees from Gilead. C. W. H. contracts for clinical research, paid to Hopkins and unrelated to this work, from Gilead, Merck, and ViiV Healthcare; consulting fees from Gilead (scientific Advisory Board, travel support, paid to author), Merck (scientific Advisory Board, travel support and honoraria, paid to author), and ViiV Healthcare (scientific Advisory Board, travel support and honoraria, paid to author); US patents (2 issued patents related to HIV prevention technology); and a leadership role as founder of Prionde Biopharma, LLC (company to develop human immunodeficiency virus (HIV) prevention product). P. J. reports grants or contracts unrelated to this work: T32 GM007546 from the National Institute of General Medical Sciences (NIGMS). R. G. reports grants or contracts unrelated to this work from Gilead Sciences: employer, UCSF, has received funding to support work as a site investigator for clinical trials related to preexposure prophylaxis using agents other than FTC/TDF; and payment for expert testimony from the US Department of Justice (related to a patent held by the US Government related to preexposure prophylaxis using tenofovir esters and emtricitabine). C. L. C. reports consulting fees as a scientific advisor to Merck and Gilead Sciences. J. M. B. reports grants to institution, unrelated to this work, from NIH and BMGF and is an employee of Gilead Sciences (salary, stock, options). J. M. reports consulting fees and participation on Scientific Advisory Board from Merck and Gilead Sciences and is treasurer and board member for the Infectious Disease Society of America. P. A. reports contracts or grants unrelated to this work from Gilead Sciences (paid to institution); and consulting fees paid to author from Gilead Sciences, Merck, and ViiV. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

## References

1. WHO. HIV/AIDS fact sheet. Available at: <https://www.who.int/en/news-room/fact-sheets/detail/hiv-aids>. Accessed 28 October 2021.
2. Grant RM, Lama JR, Anderson PL, et al. Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. *N Engl J Med* **2010**; 363:2587–99.
3. Marrazzo JM, Ramjee G, Richardson BA, et al. Tenofovir-based preexposure prophylaxis for HIV infection among African women. *N Engl J Med* **2015**; 372:509–18.
4. Baeten JM, Donnell D, Ndase P, et al. Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. *N Engl J Med* **2012**; 367:399–410.
5. Thigpen MC, Kebaabetswe PM, Paxton LA, et al. Antiretroviral preexposure prophylaxis for heterosexual HIV transmission in Botswana. *N Engl J Med* **2012**; 367:423–34.
6. Choopanya K, Martin M, Suntharasamai P, et al. Antiretroviral prophylaxis for HIV infection in injecting drug users in Bangkok. Thailand (the Bangkok Tenofovir Study): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* **2013**; 381:2083–90.
7. Karim QA, Karim SSA, Frohlich JA, et al. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. *Science* **2010**; 329:1168–74.
8. Van Damme L, Corneli A, Ahmed K, et al. Preexposure prophylaxis for HIV infection among African women. *N Engl J Med* **2012**; 367:411–22.
9. Amico KR, Stürrett MJ. Adherence to preexposure prophylaxis: current, emerging, and anticipated bases of evidence. *Clin Infect Dis* **2014**; 9:S55–60.
10. Liu AY, Hessel NA, Vittinghoff E, et al. Medication adherence among men who have sex with men at risk for HIV infection in the United States: implications for pre-exposure prophylaxis implementation. *AIDS Patient Care STDS* **2014**; 28:622–7.
11. Lindsey JC, Bosch RJ, Rudy BJ, Flynn PM. Early patterns of adherence in adolescents initiating highly active antiretroviral therapy predict long-term adherence, virologic, and immunologic control. *AIDS Patient Care STDS* **2009**; 23:799–801.
12. Rudy BJ, Murphy DA, Harris DR, Muenz L, Ellen J. Prevalence and interactions of patient-related risks for nonadherence to antiretroviral therapy among perinatally infected youth in the United States. *AIDS Patient Care STDS* **2010**; 24:97–104.
13. Murphy DA, Belzer M, Durako SJ, Sarr M, Wilson CM, Muenz LR. Longitudinal antiretroviral adherence among adolescents infected with human immunodeficiency virus. *Arch Pediatr Adolesc Med* **2005**; 159:764–70.
14. Hosek SG, Siberry G, Bell M, et al. The acceptability and feasibility of an HIV pre-exposure prophylaxis (PrEP) trial with young men who have sex with men. *J Acquir Immune Defic Syndr* **2013**; 62:447–56.
15. Molina JM, Capitant C, Spire B, et al. On-demand preexposure prophylaxis in men at high risk for HIV-1 infection. *N Engl J Med* **2015**; 373:2237–46.
16. Bauer R. NONMEM users guide: introduction to NONMEM 7.4.1. Vol 53. Gaithersburg: ICON Plc, **2017**:128.
17. Ahn JE, Karlsson MO, Dunne A, Ludden TM. Likelihood based approaches to handling data below the quantification limit using NONMEM VI. *J Pharmacokinetic Pharmacodyn* **2008**; 35:401–21.
18. Lu J, Gries J-M, Verotta D, Sheiner LB. Selecting reliable pharmacokinetic data for explanatory analyses of clinical trials in the presence of possible noncompliance. *J Pharmacokinetic Pharmacodyn* **2001**; 28:343–62.
19. Collett D. Modelling survival data in medical research. Boca Raton: Chapman and Hall/CRC, **1992**.
20. Holford N. A time to event tutorial for pharmacometricians. *CPT Pharmacometrics Syst Pharmacol* **2013**; 2:e43.
21. Donnell D, Baeten JM, Bumpus NN, et al. HIV protective efficacy and correlates of tenofovir blood concentrations in a clinical trial of PrEP for HIV prevention. *J Acquir Immune Defic Syndr* **2014**; 66:340–8.
22. Kiley R, Peatfield T, Hansen J, et al. Data sharing from clinical trials—a research funder's perspective. *N Engl J Med* **2017**; 377:1990–2.
23. Garcia-Lerma JG, Otten RA, Qari SH, et al. Prevention of rectal SHIV transmission in macaques by daily or intermittent prophylaxis with emtricitabine and tenofovir. *PLoS Med* **2008**; 5:e28.
24. Patterson KB, Prince HA, Kraft E, et al. Penetration of tenofovir and emtricitabine in mucosal tissues: implications for prevention of HIV-1 transmission. *Sci Transl Med* **2011**; 3:112re4.
25. Hendrix CW. Exploring concentration response in HIV pre-exposure prophylaxis to optimize clinical care and trial design. *Cell* **2013**; 155:515–8.
26. Celum CL, Delany-Moretlwe S, Baeten JM, et al. HIV pre-exposure prophylaxis for adolescent girls and young women in Africa: from efficacy trials to delivery. *J Int AIDS Soc* **2019**; 22(Suppl 4):e25298.
27. Klatt NR, Cheu R, Birse K, et al. Vaginal bacteria modify HIV tenofovir microbicide efficacy in African women. *Science* **2017**; 356:938–45.
28. Spreen W, Ford SL, Chen S, et al. GSK1265744 pharmacokinetics in plasma and tissue after single-dose long-acting injectable administration in healthy subjects. *J Acquir Immune Defic Syndr* **2014**; 67:481–6.
29. Cottrell ML, Garrett KL, Prince HMA, et al. Single-dose pharmacokinetics of tenofovir alafenamide and its active metabolite in the mucosal tissues. *J Antimicrob Chemother* **2017**; 72:1731–40.
30. Mayer KH, Molina JM, Thompson MA, et al. Emtricitabine and tenofovir alafenamide vs emtricitabine and tenofovir disoproxil fumarate for HIV pre-exposure prophylaxis (DISCOVER): primary results from a randomised, double-blind, multicentre, active-controlled, phase 3, non-inferiority trial. *Lancet* **2020**; 396(10246):239–54.