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Research paper

Safety and Pharmacokinetics of a Tenofovir Alafenamide Fumarate-Emtricitabine based Oral Antiretroviral Regimen for Prevention of HIV Acquisition in Women: A Randomized Controlled Trial

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ABSTRACT

Background: Daily oral emtricitabine (FTC, F)/tenofovir disoproxil fumarate (TDF) combination is approved for HIV pre-exposure prophylaxis (PrEP) in men and women. Tenofovir alafenamide fumarate (TAF) is a newer, more potent prodrug of tenofovir (TFV), and in combination with FTC, has recently been approved for prevention of HIV through rectal transmission.

Methods: This Phase I, prospective, interventional, randomized study was conducted in three clinical sites: PROFAMILIA, Santo Domingo, Dominican Republic; University of Pittsburgh and Eastern Virginia Medical School. We assessed the multi-compartmental pharmacokinetics (primary outcome) and safety (secondary outcome) among HIV uninfected women randomized to F/TDF (200mg/300mg) or F/TAF (200mg/25mg; F/TAF25) (n=24) in a single dose phase (SDP) and F/TDF, F/TAF (200mg/10mg; F/TAF10), or F/TAF25 (n=75) in a multiple dose (14 daily doses) phase (MDP). We described PK parameters in plasma, peripheral blood mononuclear cells (PBMCs), and cervicovaginal (CV) and rectal fluids and tissues. ClinicalTrials.gov #NCT02904369, completed.

Findings: Recruitment for the study began on 5 October 2016. The first participant was enrolled on 6 October 2016 and the last participant completed the study 21 November 2017.

Plasma: TFV concentrations area under curve (AUC) were ~20 fold lower following F/TAF versus F/TDF. TFV-diphosphate (TFV-DP) AUC concentrations in PBMCs were 7-fold higher with F/TAF25 versus F/TDF. Median TFV-DP concentrations in vaginal tissue (4hours post last dose) were approximately 6-fold higher with F/TAF25 versus F/TDF. TFV and TFV-DP were lower with F/TAF versus F/TDF in rectal tissue. Concentrations of FTC and FTC-triphosphate (FTC-TP) were similar across matrices and treatment arms. Gastrointestinal adverse events (AEs) occurred more frequently in F/TDF users (44.0%) than in either F/TAF group (11.5 and 12.0%).

Interpretation: F/TAF was safe and well-tolerated. TFV-DP concentrations were higher in PBMCs and similar or higher (4h post dose) in female genital tract tissues for F/TAF versus F/TDF. High FTC and FTC-TP concentrations in all compartments support the potential of F/TAF as a new PrEP combination for women.

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Research in context

Evidence before this study

Tenofovir alafenamide fumarate (TAF), a newer, more potent prodrug of tenofovir (TFV), in combination with FTC (F/TAF), was recently approved for prevention of HIV through rectal transmission in men-who-have-sex-with-men (MSM) and transgender women (TGW). Data on the safety and multi-compartment pharmacokinetics (PK) of F/TAF among healthy, HIV uninfected cis-women are needed to support its efficacy evaluation as an HIV prevention method for vaginal transmission.

Added value of this study

Data from this study help to characterize and understand the systemic and local cervico-vaginal PK of F/TAF and emtricitabine/tenofovir disoproxil fumarate (F/TDF), providing insights into the safety and pharmacodynamics (PD) of oral PrEP and supporting the effectiveness evaluation of F/TAF in women.

Implications of all the available evidence

Young healthy women have previously had difficulty with adhering to daily F/TDF for HIV pre-exposure prophylaxis (PrEP), for a variety of reasons, with some of the Phase II/III trials showing no statistically significant decrease in HIV incidence. The data presented in this study support that F/TAF has less gastrointestinal side effects than F/TDF during initiation, while offering high mucosal and systemic concentrations of the active metabolites of TFV and FTC, TFV diphosphate (TFV-DP) and FTC triphosphate (FTC-TP), compatible with HIV protection.

Introduction

Oral pre-exposure prophylaxis (PrEP) is recommended as an additional prevention option for HIV uninfected individuals at substantial risk for HIV acquisition.[1] Globally, about 40% of new HIV infections are among cis-women (hereafter women), while in sub-Saharan Africa, women account for 59% of new HIV infections among adults.[2] HIV disproportionately affects adolescent girls and young women (AGYW) largely because of vulnerabilities created by unequal cultural, social and economic status.[2] Although daily oral PrEP has been shown to be safe and effective [3–5] in men and women, there are substantial barriers to use, including gastrointestinal (GI) side effects, burdensome daily pill regimen, large pill size, stigma associated with medication use, and lack of partner or family support, which may even result in violence.[6] These challenges, among others, have made it difficult for AGYW to uptake and adhere to the limited, approved existing HIV prevention methods.[7,8] Of note, the United States (US) Centers for Disease Control and Prevention (CDC) recommends 20 days of daily oral PrEP use to ensure full protection from HIV exposure for women whose primary exposure is vaginal intercourse.[9] After this initial dosing period, pharmacokinetic (PK)/pharmacodynamics (PD) modeling data support that women likely need to take 6 – 7 doses of emtricitabine (F, FTC) combined with tenofovir disoproxil fumarate (TDF) per week in order to achieve optimal mucosal protection against cervicovaginal (CV) acquisition of HIV.[10] Safer and more potent oral PrEP regimens that use smaller pill size, lower doses, have fewer side effects, and may be more forgiving, i.e. allowing for missed doses, may help support uptake and adherence, particularly in AGYW.

TDF, in combination with FTC (F/TDF) is approved as Truvada® (Gilead Sciences, Foster City, CA) for both treatment and prevention of HIV acquisition.[3–5] Tenofovir alafenamide fumarate (TAF) is another antiretroviral (ARV) prodrug of tenofovir (TFV) with improved safety and PK properties over TDF.[11–14] TAF 25 mg, in combination with FTC 200 mg (F/TAF 25), has also been approved by the US Food and Drug Administration (FDA) as Descovy® (Gilead Sciences, Foster City, CA) for the treatment of HIV infection and for HIV prevention in individuals who are at-risk for sexually acquired HIV other than through vaginal intercourse.[15] Unlike F/TDF, there is no current approval of F/TAF 25 for the prevention of vaginal acquisition of HIV-1 among healthy women. TAF is more potent than TDF,[16] which results in significantly higher active metabolite concentrations in lymphoid cells and tissues, and demonstrates higher antiviral activity in target cells with less long-term toxicity in tissues, particularly kidney and bone.[17–21] Therefore, an oral PrEP regimen based on TAF may be safer and potentially more forgiving of imperfect adherence for HIV prevention.[15,22] The active metabolites of TFV and FTC, TFV-diphosphate (TFV-DP) and FTC-triphosphate (FTC-TP), compete with endogenous deoxyadenosine triphosphate (dATP) and deoxycytidine triphosphate (dCTP) for incorporation into the proviral DNA of HIV resulting in strand termination and determining the activity of the two drugs.[23,24]

The current study evaluated the short term safety and systemic and genital tract PK of two oral forms of daily F/TAF based regimens compared to F/TDF in women for up to 2 weeks of dosing. Two doses of F/TAF [F/TAF 200 mg/10mg (F/TAF10) and F/TAF 200 mg/25mg (F/TAF25)] were compared with the current dose of F/TDF (200mg/300 mg). Our hypothesis was that F/TAF would result in lower TFV systemic exposure and higher systemic and local intracellular and mucosal levels of the active metabolite (TFV-DP), thus maintaining or enhancing antiviral efficacy, while also improving the safety profile.

Methods

Study design

This Phase I, prospective, PK/PD study measured the systemic, cervico-vaginal (CV) and rectal PK and PD after a single dose and multiple doses of three oral tablets: F/TAF10 (multiple dose phase, MDP); F/TAF25 (single dose phase (SDP) and MDP); and F/TDF (SDP and MDP). The study was conducted in three clinical sites and reviewed and approved by the PROFAMILIA Ethics Committee, Santo Domingo, Dominican Republic; University of Pittsburgh (UPITT) Institutional Review Board (IRB) (PRO16080546), Pittsburgh, PA; and Chesapeake IRB, Columbia, MD with a waiver of oversight from Eastern Virginia Medical School (EVMS) (PRO00018534), and was registered with ClinicalTrials.gov #NCT02904369. All participants provided written informed consent prior to any study procedures. Two of the three clinical sites and the bioanalytical lab were inspected by the US FDA in 2019 with no major findings reported.

Participants

Healthy, non-pregnant, HIV-uninfected women aged 18-50 years with regular menstrual cycles, a body mass index $\geq$ 18 and $<$ 35 kg/m^2, and who were either sexually abstinent or in a monogamous relationship with a healthy partner, were eligible for the study.

Randomization and masking

This was a prospective, randomized trial. To the extent possible, given that F/TDF and F/TAF pills look different, investigators were blinded to study treatment and did not see which pill the patient received. Participants and study coordinators knew which pill was given. The laboratories and statistical/data analysts were blinded to study treatment until after database lock. For the single dose phase (SDP), described below, participants were randomized to either F/

TDF or F/TAF25 in a 1:1 ratio. The random sequences were created in block sizes of 4 using the PLAN procedure in SAS version 9.4. The electronic file containing the treatment assignments was maintained in a secure folder that only designated un-blinded data management personnel had access to until after database lock. Un-blinded data management personnel generated paper randomization envelopes with inserts containing the sequential treatment assignments and the sealed envelopes were sent to the EVMS site. To conceal the allocation procedure, random assignments were contained within the sequentially numbered, sealed opaque envelopes in a secure location at the EVMS site and opened by site study staff at the time of randomization. Treatment assignments were coded as A and B and the treatment group description was kept blinded until after database lock.

For the multiple dose phase (MDP), described below, participants were randomized to either F/TDF or F/TAF25 or F/TAF10 in a 1:1:1 ratio, stratified by site so that each site had equal sizes per treatment arm. The random sequences were created in block sizes of 6 for each site using the PLAN procedure in SAS version 9.4. The electronic file containing the treatment assignments were maintained in a secure folder that only designated un-blinded data management personnel had access to until after database lock. To conceal the allocation procedure, random assignments were accessed through Medrio by site study staff at the time of randomization. Treatment assignments were coded as X, Y, and Z and the treatment group description was kept blinded until after database lock.

Procedures

There were two phases of this study; the SDP followed by the MDP. The SDP, conducted only at the EVMS site, consisted of a screening visit and then administration of a single oral dose of F/TDF or F/TAF25 in the clinic under direct observation at visit 2. Blood samples were then collected at eight time points after the single dose (0.5, 1, 2, 4, 8, 24, 48, and 72 hours) for PK for each participant. CV fluid, rectal fluid, and CV tissue were collected (for PD and PK) at 4 hours after the single dose. There was a wash out period of at least 30 days between dosing in the SDP and the beginning of the MDP.

All three sites participated in the MDP, which consisted of 9 visits and a follow up phone call over approximately two months (Supplemental Materials, Supplemental Table 1, Schedule of Evaluations MDP). Safety assessments were performed throughout the study. PD and PK assessments were collected at baseline and at follow up visits. PK was assessed after the first dose in blood samples collected at five time points (0.5, 1, 2, 4 and 8 hours) and in CV fluid and rectal fluid collected at four time points (1, 2, 4 and 8 hours) at visit 2Mb. Participants were instructed to continue to take one dose each day for a total of 14 doses. They returned on days 2 (visit 3M), 7 (visit 4M), and 14 (visit 5M) (24 hours, 6 days, and 13 days after first dose) for predose trough blood, and CV and rectal fluid samples for PK. After trough sampling, participants took the day's dose in the clinic under direct observation. After the final (14^{th}) dose at visit 5M, PK was assessed in blood samples collected at 0.5, 1, 2, 4, 8, 24, 48, and 72 hours, and in CV and rectal fluid collected at 4, 8, 24, 48 and 72 hours. To evaluate multiple time points, cervical and vaginal tissue for PK were collected at 4, 24 and 48 hours after the last dose at EVMS, UPITT, and Profamilia, respectively. Rectal biopsies were collected for PK 4 hours after the final dose at UPITT. All study drugs were donated by Gilead Sciences, Inc. (Foster City, CA, USA).

Outcomes

Primary objective: Pharmacokinetics

All drug concentrations were quantified in all matrices by a single PK laboratory (University of North Carolina), by liquid chromatography tandem mass spectrometry (LC-MS/MS) methods using isotopically labeled internal standards and detected on an AB Sciex API-5000 triple quadrupole mass spectrometer (see supplemental methods for details). Whole blood was collected into ethylenediaminetetraacetic acid (EDTA) tubes, processed by centrifugation at 4°C and resulting plasma was stored at -80°C for quantification of TAF, TFV and FTC. Whole blood was collected into BD Vacutainer CPT tubes and processed for PBMCs as previously described.[10] Resulting PBMCs were counted on a hemocytometer or automated cell counter, lysed in 70:30 methanol water and stored at -80°C for quantification of TFV-DP, FTC-TP, dATP, and dCTP. CV fluid was collected by holding a sponge (Merocel eye-wick Spears) in place for 60 seconds against the ectocervix and vaginal wall, respectively. To collect rectal fluid, a sponge was introduced through an anoscope to visualize rectal mucosa and held on the mucosa for 60 seconds. The sponges were placed in cryovials and stored at -80°C for quantification of TAF, TFV and FTC. Cervical and vaginal biopsies (weighing ≈20 mg/each specimen) were collected using a Tischler forceps and snap frozen in liquid nitrogen immediately after sampling and stored at -80°C. Rectal biopsies (weighing 15-25 mg each) were collected at UPITT only using a flexible sigmoidoscope with radial jaw #4 jumbo without needle and snap frozen in liquid nitrogen immediately after sampling and stored at -80°C. TAF, TFV, TFV-DP, FTC, FTC-TP, dATP and dCTP were quantified in tissues. Additional information on the PK methods is detailed in Supplemental Methods section.

Primary objective: Pharmacodynamics

Ex-vivo modeling of mucosal HIV infection was assessed in the cervical and vaginal tissue obtained 4 hours after the last dose at EVMS and in the rectal tissue collected 4 hours after the last dose at UPITT. Anti-herpes simplex virus type 2 and anti-HIV activity PD was also assessed in CV and rectal fluid collected at 4 hours (all sites), 24 hours (UPITT), and 48 hours (Profamilia) after the final dose. The results of the PD analyses will be reported in a separate manuscript.

Secondary objective: Safety

Safety was primarily assessed by treatment emergent AEs (TEAEs), and changes from baseline, pre-product use to post product use in clinical laboratory tests, including complete blood count, serum chemistries and lipids, physical examination, and pelvic examination. We also noted any concomitant medication use. The safety analysis included all participants having at least one product use. AEs were collected at each study visit after genital sampling at baseline, graded for severity based on the NIH/NIAID Division of AIDS (DAIDS) severity scale. The relationship to study product or study procedure was noted and coded using the Medical Dictionary for Regulatory Activities (MedDRA). TEAEs and laboratory AEs that represented an increase in severity from baseline based on the NIH/NIAID DAIDS severity scale are presented.

Statistical analysis

Sample size was based on feasibility, and was estimated to be sufficient to describe parameter estimates of PK properties and safety assessments in this Phase I study; thus, the analysis was primarily descriptive and graphical. In general, continuous variables were summarized to indicate the population sample size (N), number of participants with available data (n), median, minimum, and maximum values. Categorical variables were summarized by the population size, number of participants with available data, number of participants in each category, and the percentage of participants in each category. Unless otherwise noted, the denominator to determine the percentage of participants in each category was based on the number of participants with available data. Adverse events (AEs) were compared based on treatment group by Fisher exact test-or Pearson Chi-square test, depending on cell size. Statistical significance testing was two-sided and performed using α=0.05.

PK analysis included descriptive statistics for TAF, TFV, and FTC concentrations for all plasma, tissue, and fluid sample types, in addition to TFV-DP, FTC-TP, competing nucleotides dATP and dCTP concentrations for peripheral blood mononuclear cells (PBMCs), and CV and rectal tissue samples. PK concentrations were summarized for the SDP and the MDP by treatment group and sampling time point using descriptive statistics, to include median and range. PK parameters were estimated for plasma and PBMC concentrations by non-compartmental methods. PK parameters were summarized by treatment group using descriptive statistics that included the coefficient of variation. Summaries of the median (range) maximum concentration (Cmax), time to maximum concentration (T_{max}), maximum concentration at 24 hours (C_{24h}) and area under the curve between dosing and 24 hours (AUC_{0-24}), calculated by linear trapezoidal summation, are included for PK parameters in plasma and PBMCs. For CV tissue concentrations where median sampling computations were used, only the PK median (minimum, maximum), lower limit of quantification (LLOQ) and proportion of samples with concentrations below the limit of quantification (BLQ) are presented. For calculation of descriptive statistics, the value of one half of the LLOQ was imputed for all samples that had a reported concentration of BLQ. Otherwise no other imputations were made.

Role of the funding source

The funders (USAID) were not involved in the study design, but were given regular reports on the study conduct. Gilead Sciences donated the study products and were given the opportunity to provide input on the study design and the final manuscript. USAID and Gilead Sciences had no role in the conduct of the study, data collection, data analysis, data interpretation, or writing of the main report. The corresponding author (AT) had full access to all the data after database lock. The principal investigator (GD) had final responsibility for the decision to submit for publication.

Results

The first participant was enrolled on 6 October 2016 and the last participant completed the study 21 November 2017. Twenty five women were screened in SDP and 87 additional women were screened in MDP (figure 1). Twenty-four women were enrolled and treated in the SDP; 22 of these women continued to the MDP and 53 additional women were enrolled and treated in the MDP for a total of 75 in the MDP (figure 1). No participants discontinued the study due to AEs. All participants in both phases are included in all the analysis populations. Compliance for the SDP was 100% as the single dose was administered on site. Based on self-report, all but 3 participants were

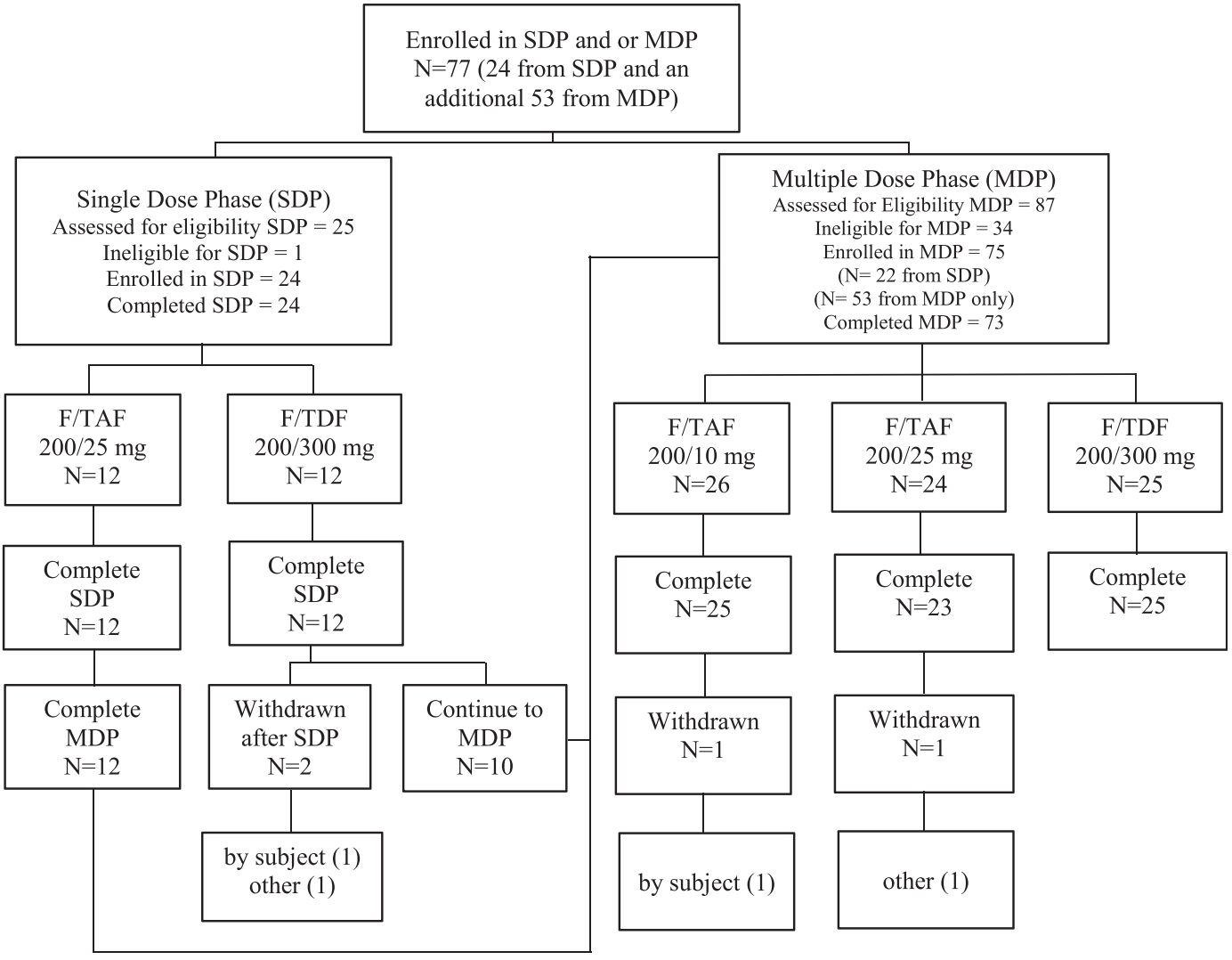


Figure 1. F/TAF = emtricitabine + tenofovir alafenamide; F/TDF = emtricitabine + tenofovir disoproxil fumarate; MDP = Multiple Dose Phase; SDP = Single dose Phase

Table 1
Baseline characteristics

	Single Dose			Multiple Dose			
	F/TAF (200/25) n=12	F/TDF (200/300) n=12	Total n=24	F/TAF (200/10) n=26	F/TAF (200/25) n=24	F/TDF (200/300) n=25	Total n=75
Age, Mean (SD)	34.1 (7)	37.7 (7)	35.9 (7)	33.2 (7)	34.6 (8)	32.8 (9)	33.5 (8)
Body-mass index, kg/m^2 Mean (SD)	28.3 (4)	26.7 (4)	27.5 (4)	26.7 (4)	27.0 (4)	26.8 (4)	26.8 (4)
Race and Ethnicity N (%)*							
Hispanic or Latino	1 (8)	2 (17)	3 (13)	8 (31)	9 (38)	10 (40)	27 (36)
American Indian or Alaska Native	0 (0)	1 (8)	1 (4)	0 (0)	0 (0)	1 (4)	1 (1)
Asian	0 (0)	1 (8)	1 (4)	2 (8)	0 (0)	1 (4)	3 (14)
Black or African American	6 (50)	1 (8)	7 (29)	15 (58)	12 (50)	13 (52)	40 (53)
White	6 (50)	10 (83)	16 (67)	18 (69)	20 (83)	18 (72)	56 (75)
Contraceptive Method used in study N (%)*							
Sterilization	3 (25)	4 (33)	7 (29)	12 (46)	11 (46)	11 (44)	34 (45)
Abstinence	5 (42)	5 (42)	10 (42)	5 (19)	3 (13)	10 (40)	18 (24)
Combination Hormonal Contraception	5 (42)	4 (33)	9 (38)	6 (23)	6 (25)	1 (4)	13 (17)
Condoms	1 (8)	3 (25)	4 (17)	4 (15)	1 (4)	4 (16)	9 (12)
Copper IUD	1 (8)	0 (0)	1 (4)	0 (0)	5 (21)	1(4)	6 (8)
Same sex relationship	0 (0)	0 (0)	0 (0)	1 (4)	0 (0)	0 (0)	1 (1)

* Subjects reporting more than one race or contraceptive method are included in all relevant categories. Percentages may add up to >100%.

Table 2
Overall summary of treatment emergent adverse events

	Single Dose Phase		Multiple Dose Phase		
	F/TAF (200/25 mg) (N=12)	F/TDF (200/300 mg) (N=12)	F/TAF (200/10 mg) (N=26)	F/TAF (200/25 mg) (N=24)	F/TDF (200/300 mg) (N=25)
Total Number of TEAEs	9	8	21	31	49
Total Number of TESAEs	0	0	0	0	1
Number (%) of Participants Reporting at Least One:					
TEAE	5 (42)	4 (33)	13 (50)	18 (75)	20 (80)
TEAE by Severity[1]					
Grade 1: Mild	3 (25)	1 (8)	9 (35)	8 (33)	10 (40)
Grade 2: Moderate	1 (8)	2 (17)	4 (15)	10 (42)	8 (32)
Grade 3: Severe	1 (8)	1 (8)	0 (0)	0 (0)	1 (4)
Grade 4: Potentially Life-Threatening	0 (0)	0 (0)	0 (0)	0 (0)	1 (4)
Grade 5: Death	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Grade ≥ 2	2 (17)	3 (25)	4 (15)	10 (42)	10 (40)
Grade ≥ 3	1 (8)	1 (8)	0 (0)	0 (0)	2 (8)
TEAE by Relationship[2]					
Not Related	5 (42)	3 (25)	9 (35)	12 (50)	8 (32)
Related	0 (0)	1 (8)	4 (15)	6 (25)	12 (48)
TEAE with Grade ≥ 3 Related to Study Drug	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Gastrointestinal TEAE	1 (8)	1 (8)	3 (12)	3 (13)	11 (44)
TESAE	0 (0)	0 (0)	0 (0)	0 (0)	1 (4)

TEAE=treatment-emergent adverse event; TESAE=treatment-emergent serious adverse event
[1] Participants reporting more than one adverse event were counted only once using the highest severity.
[2] Participants reporting more than one adverse event were counted only once using the closest relationship to study drug (i.e., "Related" or "Not Related").

100% compliant with the treatment regimen during the MDP: one participant in the F/TAF 25 group and 2 participants in the F/TDF group each reported missing one dose out of 14 (92.9% compliant). The demographic characteristics of participants in both the SDP and MDP are listed in table 1.

In the SDP, five participants (41.7%) in the F/TAF25 group reported 9 TEAEs and 4 (33.3%) in the F/TDF group reported 8 TEAEs (p = 1.00) (table 2). Only one product related TEAE of diarrhea was reported in the SDP (F/TDF group). The most common TEAE in the SDP was biopsy related procedural pain (3 participants in the F/TAF25 group). In the MDP, 13 participants (50.0%) in the F/TAF10 group, 18 (75.0%) in the F/TAF25 group and 20 (80.0%) in the F/TDF group reported 21, 31 and 49 TEAEs, respectively (p = 0.048) (table 2). Gastrointestinal (GI) TEAEs occurred more frequently in the F/TDF group (44.0%) than in either F/TAF group (11.5% and 12.0%) (p = 0.016). The most common TEAE was nausea, which was more frequent in the F/TDF group (8 [32.0%]) than in the F/TAF10 group (1 [3.8%]) or F/TAF25 group (2 [8.3%]) (p = 0.020). GI AEs reported by 6 (12.0%) participants in the F/TAF groups were of mild intensity except for one moderate event of nausea in the F/TAF10 group. Of the 11 (44.0%) participants in the F/TDF group reporting GI AEs, 6 (24.0%) reported at most mild AEs, 4 (16.0%) reported moderate AEs and 1 (4.0%) reported severe upper abdominal pain. Of the participants who experienced AEs, most were reported as mild or moderate (table 2); only 2 participants, both in the F/TDF group, reported AEs of Grade ≥ 3, a Grade 3 event of upper abdominal pain and a Grade 4 event of hemorrhage after tissue biopsy (table 2).

Abnormal post treatment blood laboratory values occurred in 5 [19.2%], 10 [41.7%], and 8 [32.0%] participants in the F/TAF 10, F/TAF 25, and F/TDF groups, respectively (p = 0.22). The most common type

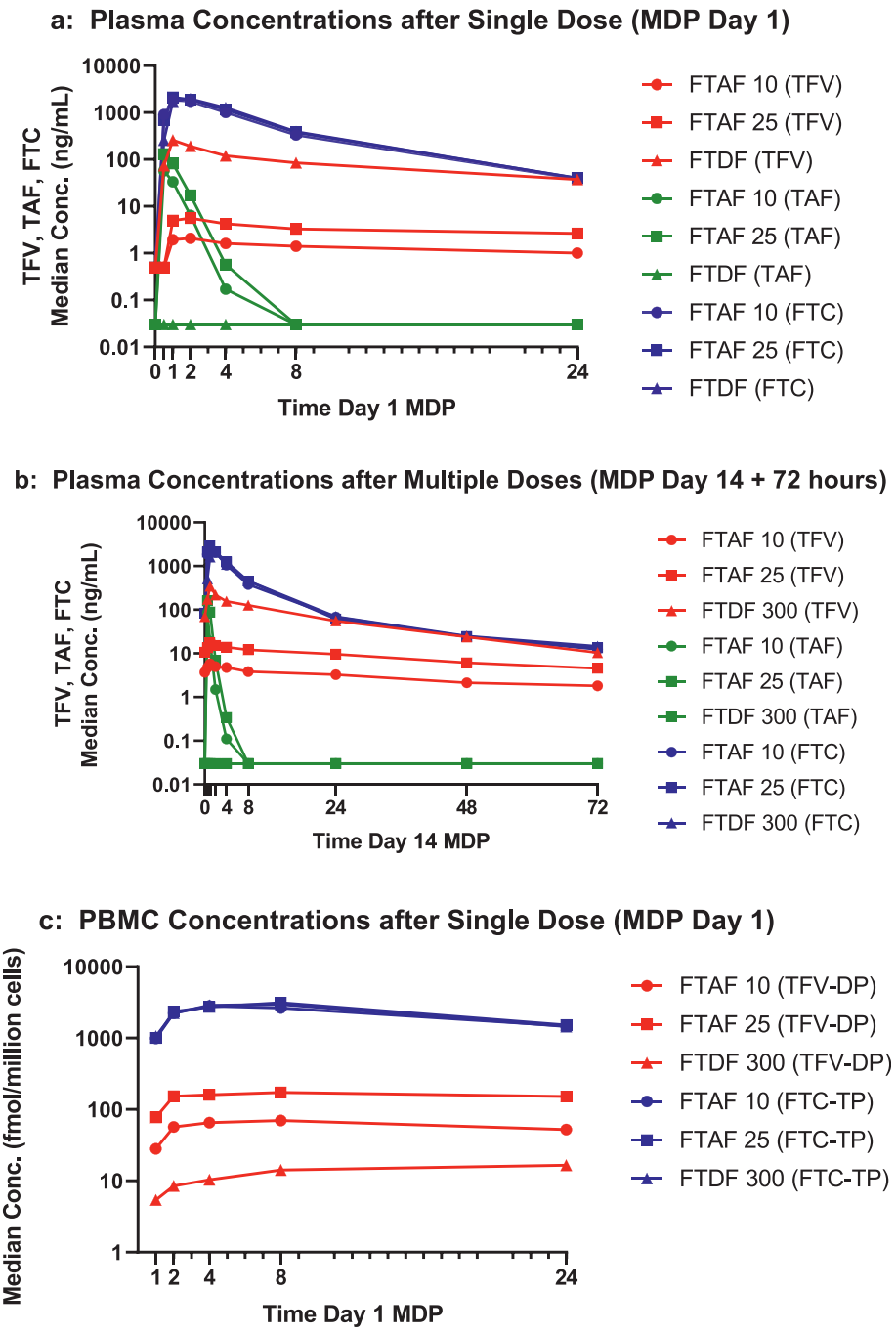


Figure 2a. Red circle = FTAF 10 (TFV); Red square = FTAF 25 (TFV); Red triangle = FTDF (TFV); Green circle = FTAF 10 (TAF); Green square = FTAF 25 (TAF); Green triangle = FTDF (TAF); Blue circle = FTAF 10 (FTC); Blue square = FTAF 25 (FTC); Blue triangle = FTDF (FTC)

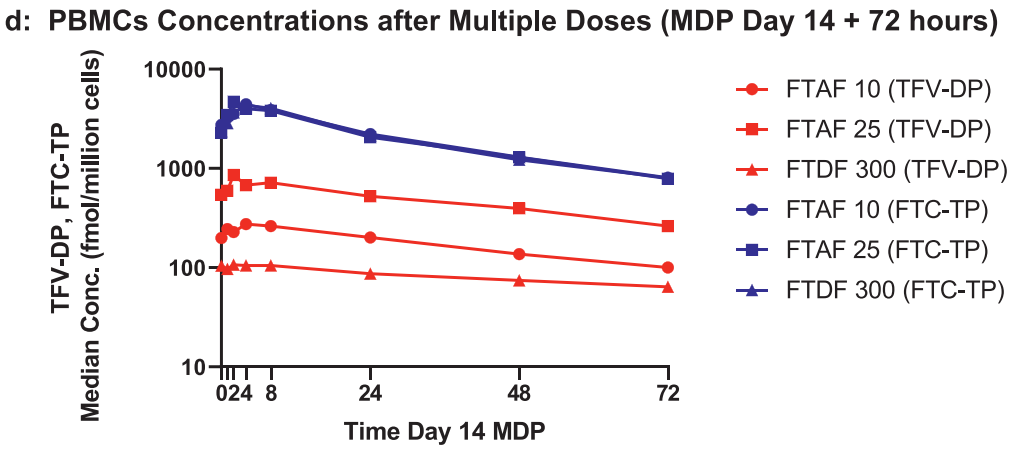
Figure 2b: Red circle = FTAF 10 (TFV); Red square = FTAF 25 (TFV); Red triangle = FTDF (TFV); Green circle = FTAF 10 (TAF); Green square = FTAF 25 (TAF); Green triangle = FTDF (TAF); Blue circle = FTAF 10 (FTC); Blue square = FTAF 25 (FTC); Blue triangle = FTDF (FTC)

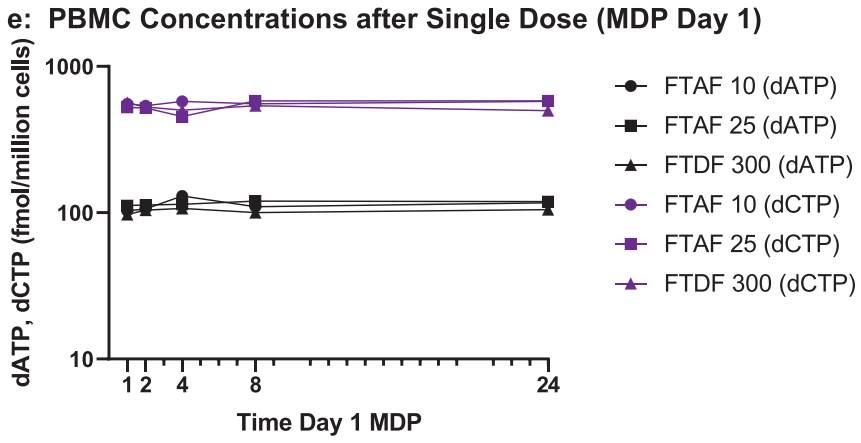
Figure 2c: Red circle = FTAF 10 (TFV-DP); Red square = FTAF 25 (TFV-DP); Red triangle = FTDF (TFV-DP); Blue circle = FTAF 10 (FTC-TP); Blue square = FTAF 25 (FTC-TP); Blue triangle = FTDF (FTC-TP)

Figure 2d: Red circle = FTAF 10 (TFV-DP); Red square = FTAF 25 (TFV-DP); Red triangle = FTDF (TFV-DP); Blue circle = FTAF 10 (FTC-TP); Blue square = FTAF 25 (FTC-TP); Blue triangle = FTDF (FTC-TP)

Figure 2e: Black circle = FTAF 10 (dATP); Black square = FTAF 25 (dATP); Black triangle = FTDF (dATP); Purple circle = FTAF 10 (dCTP); Purple square = FTAF 25 (dCTP); Purple triangle = FTDF (dCTP)

Figure 2f: Black circle = FTAF 10 (dATP); Black square = FTAF 25 (dATP); Black triangle = FTDF (dATP); Purple circle = FTAF 10 (dCTP); Purple square = FTAF 25 (dCTP); Purple triangle = FTDF (dCTP)





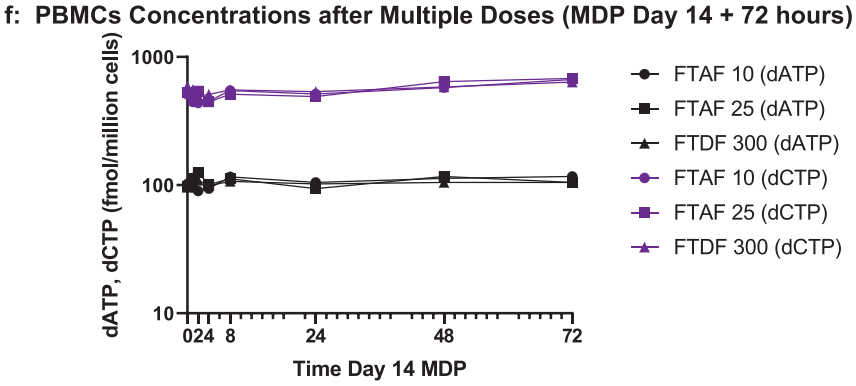


Figure 2a. Continued.

of individual abnormal value, occurring in 5-12.5% of participants in at least one treatment group, included decreases in creatinine clearance, hemoglobin and sodium and increases in triglycerides, cholesterol, glucose and potassium. However, these mostly mild and relatively infrequent individual laboratory AEs did not display any noticeable or meaningful clinical pattern based on study drug.

In general, single-dose concentrations demonstrated similar values in all biomatrices in both the SDP and on day 1 of 14 in the MDP. Sampling times were the same after the SDP and day 1 of the MDP for plasma and PBMCs. Therefore we describe the results of these two datasets together, although we present data separately both in main and supplemental tables and figures.

TAF was quantifiable in plasma for up to 8 hours following the administration of a single dose and multiple (14) doses of F/TAF and was not quantifiable in any samples following administration of F/TDF, as expected (figures 2a – 2b). TFV plasma Cmax was 60- and 20-fold lower following multiple doses of F/TAF10 and 25 compared to F/TDF, respectively (figure 2b, table 3). After a single dose, concentrations of TFV in plasma were much lower following administration of F/TAF versus F/TDF (figure 2a, table 3). Median plasma TFV concentrations at the pre-dose trough on day 7 (visit 4M) were 3.20 ng/mL, 9.16 ng/mL and 73.60 ng/mL for F/TAF10 F/TAF25 and F/TDF respectively. Concentrations of FTC were similar in plasma following single and multiple doses of all three products (figures 2a, 2b, table 3).

Concentrations of TFV-DP in PBMCs were similar in the SDP and after the first dose in the MDP (figure 2c, table 3). Median TFV-DP C_{max} concentrations in PBMC were higher following a single dose of F/TAF25 compared to concentrations after multiple doses of F/TDF

Table 3
Tenofovir and emtricitabine pharmacokinetic parameters in plasma and peripheral blood mononuclear cells

Biomatrix and Analyte	PK Paramater	Single Dose Phase (SDP) (Median, Range) (n = 24)		After First Dose MDP (Median, Range) (n = 75)			Multiple-Dose Phase (MDP) Values after 14 Daily Doses Median (Range)		
		F/TAF 200/25 mg (N = 12)	F/TDF 200/300 (N = 12)	F/TAF 200/10 mg (N=26)	F/TAF 200/25 mg (N = 24)	F/TDF 200/300 mg (N = 25)	F/TAF 200/10 mg (N=26)	F/TAF 200/25 mg (N = 24)	F/TDF 200/300 mg (N = 25)
Plasma TFV	$AUC_{0\text{-}24}$ (h•ng/mL)	77 (39, 224)	2260 (1281, 2852)	31 (22, 56)	77 (50.9, 286.2)	1959 (1432, 2906)	101 (21, 167)	294 (178, 671)	2943 (1371, 4014)
	C_{max} (ng/mL)	6.2 (4, 17)	436 (272, 625)	2.4 (1, 4)	5.8 (4, 28)	306 (158, 499)	6 (2, 11)	17 (11, 39)	357 (176, 627)
	C_{24h} (ng/mL)	2.3 (1, 7)	37.2 (22, 59)	1 (1, 2)	2.7 (1, 9)	37.3 (25, 61)	3.3 (1, 6)	9.6 (5, 23)	56 (26, 76)
	Tmax (hrs)	1.5 (1, 2)	1 (1, 2)	2 (1, 4)	2 (1, 4)	1 (1, 2)	1 (1, 4)	1 (1, 4)	1 (1, 2)
PBMC TFV-DP	$AUC_{0\text{-}24}$ (h•fmol/10^6 cells)	3,504 (1565, 10,009)	430 (180, 842)	1389 (681, 2977)	3781 (2058, 8288)	341 (186, 1505)	6,078 (961, 11604)	15,215 (8700, 42,689)	2,498 (562, 3993)
	C_{max} (fmol/10^6 cells)	184 (80, 741)	33 (13, 60)	74 (43, 149)	195 (117, 440)	23 (9, 129)	314 (50, 18029)	1021 (444, 5726)	139 (26, 1000)
	C_{24h} (fmol/10^6 cells)	118 (50, 397)	26 (10, 39)	52.3 (12, 149)	152 (1, 350)	16.5 (8, 41)	201 (30, 395)	523 (273, 931)	87 (25, 141)
	Tmax (hrs)	4 (2, 8)	24 (2, 72)	4 (2, 24)	8 (2, 24)	24 (1, 24)	4 (1, 72)	2 (1, 48)	4 (0, 48)
Plasma FTC	$AUC_{0\text{-}24}$ (h•fmol/10^6 cells)	11172 (8943, 22663)	12464 (9410, 16136)	10877 (7826, 20993)	13215 (9601, 25861)	12317 (9030, 18349)	13824 (8232, 19836)	15048 (11017, 22847)	13238 (8280, 20388)
	C_{max} (fmol/10^6 cells)	2165 (1750, 3950)	2105 (1270, 3370)	2270 (1370, 3440)	2130 (1510, 4020)	2060 (1300, 3740)	2480 (1140, 4050)	2950 (1580, 4240)	2190 (1110, 3670)
	C_{24h} (ng/mL)	34.0 (15, 59)	40.5 (24, 65)	38.5 (18, 59)	40.1 (25, 86)	39.7 (22, 58)	68.2 (23, 106)	61 (39, 174)	69.4 (37, 144)
	Tmax (hrs)	1 (1, 2)	1 (1, 2)	1 (1, 2)	1.5 (1, 4)	2 (1, 4)	1 (1, 2)	1 (1, 2)	2 (1, 4)
PBMC FTC-TP	$AUC_{0\text{-}24}$ (h•fmol/10^6 cells)	67621 (47260, 87037)	59845 (29354, 79636)	52718 (29589, 97269)	56535 (38445, 80424)	54894 (28850, 98685)	86484 (26672, 189710)	80887 (57843, 173622)	78228 (49154, 187278)
	C_{max} (fmol/10^6 cells)	4265 (2475, 6434)	3595 (1750, 5339)	3120 (1705, 9464)	3236 (2187, 5483)	3403 (1634, 6884)	5116 (2061, 107133)	5149 (3196, 26924)	4516 (2806, 23788)
	C_{24h} (fmol/10^6 cells)	1662 (1029, 2038)	2055 (963, 2339)	1475 (616, 3170)	1518 (24, 2634)	1448 (745, 3508)	2219 (994, 4909)	2060 (1313, 3940)	2145 (1208, 4813)
	Tmax (hrs)	4 (2, 8)	4 (2, 8)	4 (2, 8)	8 (2, 8)	8 (1, 8)	4 (0, 72)	2 (1, 8)	4 (1, 8)

(figures 2c, 2d, table 3). Similarly, a single dose of F/TAF25 achieved a TFV-DP exposure (median $AUC_{0\text{-}24}$) that was 1.5-fold higher than following 14 daily doses of F/TDF (table 3). TFV-DP PBMC Cmax was 2- and 7-fold higher following multiple doses of F/TAF10 and 25 compared to F/TDF, respectively (table 3). At the pre-dose trough on day 7, median TFV-DP concentrations in PBMCs were 177 fmol/10^6 cells, 508 fmol/10^6 cells and 73 fmol/10^6 cells for F/TAF10, F/TAF25 and F/TDF respectively. Concentrations of FTC-TP in PBMCs were generally similar among all three product groups after a single dose and multiple doses (figures 2c, 2d, table 3), as were the endogenous nucleotides dATP and dCTP (figures 2e – 2f).

TAF was not quantifiable in any cervical, vaginal or rectal tissue samples with an average LLOQ of 1.11 ng/g, 0.95 ng/g, and 1.45 ng/g, respectively. Concentrations of endogenous nucleotide dATP in cervical and vaginal tissue were similar among the three treatment groups and with medians ranging from 112,771 – 324,704 fmol/g between 4 and 48 hours post the 14th dose of each treatment (table 4). dCTP in cervical and vaginal tissue was also similar between the 3 treatments with medians ranging from 88,076 – 251,617 fmol/g between 4 and 48 hours post the 14th dose (table 4).

TFV concentrations in cervical and vaginal tissue were generally lower at 4 and 24 hours following 14 daily doses with F/TAF10 or F/TAF25 than with F/TDF, although TFV values were similarly low in all groups at 48 hours post MDP (figure 3a, table 4). In spite of lower TFV concentrations, median concentrations of TFV-DP in cervical and vaginal tissue 4 hours after multiple dosing (Day 14) were approximately 3 to 6-fold higher following treatment with F/TAF25 than with F/TDF (figure 3b). Additionally, 100% of vaginal tissue samples had quantifiable TFV-DP concentrations at 4 hours after the 14th dose of F/TAF 25, versus only 38% and 22% for F/TDF and F/TAF10, respectively (table 4). The percentage of samples with quantifiable TFV-DP for F/TAF25 decreased at 24h showing similar percentages to F/TDF and were mostly unquantifiable for both products at 48h (figure 3b, table 4). TFV-DP was quantifiable in fewer samples at 4 hours after a single dose compared to 4 hours after 14 daily doses with both F/TAF25 and F/TDF (table 4). Median cervical and vaginal TFV-DP tissue concentrations were also 5 fold higher in the F/TAF25 group when compared to F/TDF, and after multiple doses when compared to after a single dose (figure 3b, table 4). FTC and FTC-TP were quantifiable in almost all tissue specimens at all times, with similar concentrations for F/TAF25 and F/TDF and higher concentrations after multiple doses compared to after a single dose (figures 3c and 3d, table 4). FTC and FTC-TP cervical and vaginal tissue concentrations were similar among the three treatment groups (figures 3c and 3d, table 4). Four hours after last dosing, median FTC concentrations were above 1000 ng/g tissue and median FTC-TP concentrations were above 10^6 fmol/g tissue (figures 3a and 3b, table 4). dATP and dCTP were quantifiable in all cervical and vaginal tissue specimens at all time points measured, with similar concentrations among the three treatment groups (table 4).

Median rectal tissue TFV concentrations were approximately 20 and 8-fold lower at 4 hours post the 14th dose of F/TAF10 and F/TAF25 respectively compared to F/TDF (figure 3e, table 4). Similarly, median rectal tissue TFV-DP concentrations 4 hours post dose were approximately 44-fold and 16-fold lower following F/TAF10 and F/TAF25, respectively compared to F/TDF (figure 3e, table 4). Median concentrations of FTC in rectal tissues were similar between the three treatment groups (figure 3f, table 4) but median FTC-TP was 5 to 6-fold higher after F/TDF compared to F/TAF10 or F/TAF25 (figure 3f, table 4). Concentrations of competing nucleotides dATP and dCTP in rectal tissue were similar between the three treatment groups (table 4).

Concentrations of TAF in cervical, vaginal and rectal fluids following a single dose of F/TAF25 and after the first dose and multiple doses of F/TAF10 or F/TAF25 were low and mostly unquantifiable. Concentrations of TFV in cervical and vaginal fluids after a single dose

Table 4
Cervical, Vaginal and Rectal Tissue Drug Concentrations – Single Dose (SDP) (PK in tissue collected 4 hours post dosing, vaginal only) or after 14th Dose (MDP)

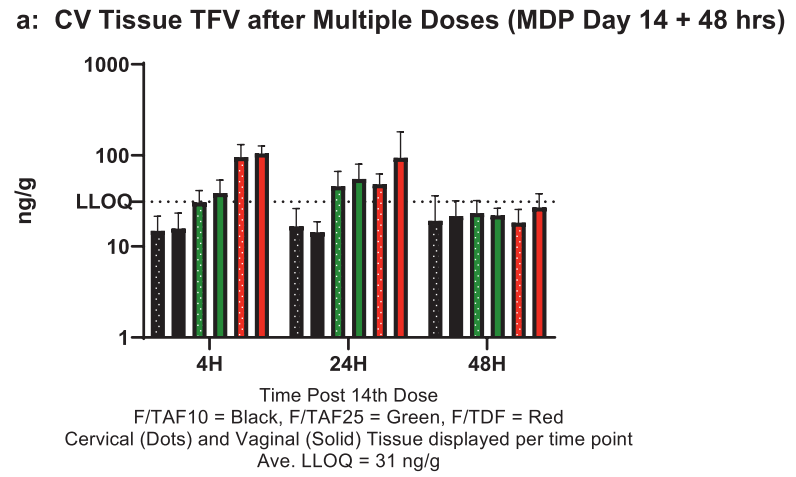
Tissue Matrix and Drug Measured	Time Point after Dose (Hours)	Variable	F/TAF10	F/TAF25		F/TDF	
			Multiple Dose Phase after 14th dose (N = 26)	Single Dose Phase (N = 12)	Multiple Dose Phase after 14th dose (N=24)	Single Dose Phase (N = 12)	Multiple Dose Phase after 14th dose (N=25)
Cervical TFV (ng/g) Average LLOQ = 30.9 ng/g	4 hours	Detectable*	1/9 (11%)	0/100 (0%)	3/8 (38%)	11/12 (92%)	7/8 (88%)
		Median (Min, Max)	13.1 (7, 29)	10.3 (4, 17)	28.4 (19, 49)	61.0 (29, 162)	87.0 (41, 149)
	24 hours	Detectable*	2/8 (25%)		7/8 (88%)		7/9 (78%)
		Median (Min, Max)	12.4 (7, 30)		41.9 (19, 85)		52.8 (24, 63)
	48 hours	Detectable*	0/8 (0%)		2/8 (25%)		2/8 (25%)
		Median (Min, Max)	14.1 (6, 60)		24.0 (13, 39)		15.3 (12, 30)
Cervical FTC (ng/g) Average LLOQ = 30.9 ng/g	4 hours	Detectable*	9/9 (100%)	12/12 (100%)	8/8 (100%)	12/12 (100%)	8/8 (100%)
		Median (Min, Max)	1222 (657, 3432)	981 (748, 1524)	1526 (1096, 1821)	1100 (519, 1454)	1270 (976, 1597)
	24 hours	Detectable*	8/8 (100%)		8/8 (100%)		9/9 (100%)
		Median (Min, Max)	249 (164, 1016)		429 (157, 1437)		351 (168, 859)
	48 hours	Detectable*	8/8 (100%)		6/8 (75%)		7/8 (88%)
		Median (Min, Max)	79 (44, 250)		78 (25, 209)		115 (20, 270)
Cervical TFV-DP (fmol/g) Average LLOQ 69,100 fmol/g	4 hours	Detectable*	1/9 (11%)	1/12 (8%)	6/8 (75%)	0/12 (0%)	1/8 (13%)
		Median (Min, Max)	30297 (16461, 63651)	25,095 (9,006, 38,951)	126297 (50797, 259921)	27,188 (7,695, 118,912)	37523 (19082, 91772)
	24 hours	Detectable*	0/8 (0%)		2/8 (25%)		2/9 (22%)
		Median (Min, Max)	22308 (14265, 54566)		41895 (22625, 111,115)		45622 (27263, 90157)
	48 hours	Detectable*	0/8(0%)		1/8 (13%)		0/9 (0%)
		Median (Min, Max)	31558 (14347, 133214)		56854 (17884, 66817)		27069 (17171, 67221)
Cervical FTC-TP (fmol/g) Average LLOQ = 63,400 fmol/g	4 hours	Detectable*	9/9 (100%)	12/12 (100%)	8/8 (100%)	12/12 (100%)	8/8/(100%)
		Median (Min, Max)	1062850 (373412, 3972748)	708,886 (260,682, 1,138,594)	1523240 (537742, 3772018)	787,268 (293,288, 2,428,866)	937184 (436712, 2448605)
	24 hours	Detectable*	8/8 (100%)		8/8 (100%)		8/9 (89%)
		Median (Min, Max)	329998 (141205, 487682)		308368 (210920, 638006)		626922 (29867, 1098206)
	48 hours	Detectable*	8/8 (100%)		7/8 (88%)		8/8 (100%)
		Median (Min, Max)	131064 (40032, 522526)		102522 (50801, 339414)		202599 (96826, 627710)
Cervical dATP (fmol/g) Average LLOQ = 20,976 fmol/g	4 hours	Detectable*	9/9 (100%)	12/12 (100%)	8/8 (100%)	12/12 (100%)	8/8 (100%)
		Median (Min, Max)	226174 (113236, 542852)	187,584 (82,135, 555,931)	256318 (136577, 569294)	228,842 (161150, 489316),	236080 (79219, 432377)
	24 hours	Detectable*	8/8 (100%)		8/8 (100%)		9/9 (100%)
		Median (Min, Max)	112771 (69076, 240991)		142893 (78959, 304544)		117853 (12599, 495068)
	48 hours	Detectable*	8/8 (100%)		8/8 (100%)		8/8 (100%)
		Median (Min, Max)	178846 (59064, 501109)		196324 (110258, 454750)		264077 (115310, 511294)
Cervical dCTP (fmol/g) Average LLOQ = 22,054 fmol/g	4 hours	Detectable*	9/9 (100%)	12/12 (100%)	8/8 (100%)	12/12 (100%)	8/8 (100%)
		Median (Min, Max)	249397 (56655, 350835)	139579 (90801, 457493)	201640 (101782, 346702)	214895 (119599, 308829)	183072 (86456, 303736)
	24 hours	Detectable*	8/8 (100%)		8/8 (100%)		9/9 (100%)
		Median (Min, Max)	88076 (42784, 136149)		111569 (50674, 211522)		106900 (13327, 276922)
	48 hours	Detectable*	8/8 (100%)		8/8 (100%)		8/8 (100%)
		Median (Min, Max)	160090 (56513, 296161)		142005 (81095, 316739)		189702 (86436, 281991)
Vaginal TFV (ng/g) Average LLOQ 30.9 ng/g	4 hours	Detectable*	1/9 (11%)	4/12 (33%)	7/8 (88%)	10/12 (83%)	8/8 (100%)
		Median (Min, Max)	14.3 (7, 27)	12.4 (5, 34)	35.6 (25, 73)	63.5 (19, 93)	101.4 (78, 151)
	24 hours	Detectable*	1/8 (13%)		6/8 (75%)		9/9 (100%)
		Median (Min, Max)	13.3 (10, 24)		48.0 (28, 103)		63.1 (26, 319)
	48 hours	Detectable*	2/8 (25%)		5/8 (63%)		4/8 (50%)
		Median (Min, Max)	21.4 (11, 40)		22.3 (17, 29)		26 (15, 49)
Vaginal FTC (ng/g) Average LLOQ 30.9 ng/g	4 hours	Detectable*	9/9 (100%)	12/12 (100%)	8/8 (100%)	8/8 (100%)	8/8 (100%)
		Median (Min, Max)	1130 (559, 1835)	941 (737, 1881)	1508 (800, 1813)	1059 (602, 1843)	1205 (606, 1811)
	24 hours	Detectable*	8/8 (100%)		8/8 (100%)		9/9 (100%)
		Median (Min, Max)	541 (134, 1805)		452 (231, 1204)		561 (131, 1138)

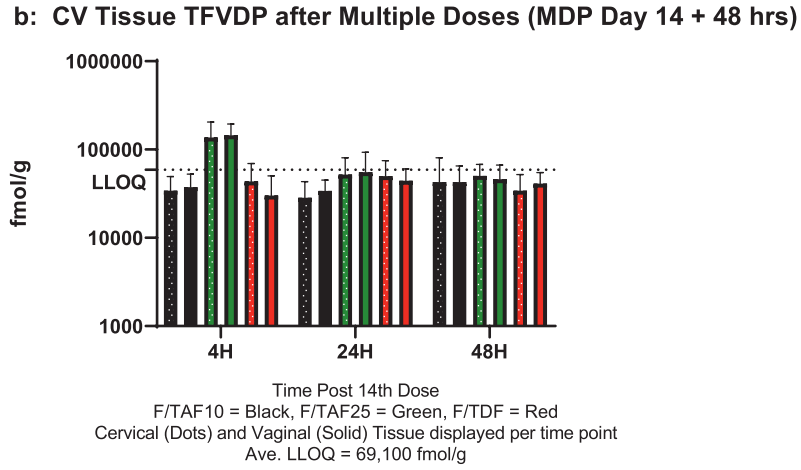
(*continued on next page*)

Table 4 (*Continued*)

Tissue Matrix and Drug Measured	Time Point after Dose (Hours)	Variable	F/TAF10	F/TAF25		F/TDF	
			Multiple Dose Phase after 14th dose (N = 26)	Single Dose Phase (N = 12)	Multiple Dose Phase after 14th dose (N=24)	Single Dose Phase (N = 12)	Multiple Dose Phase after 14th dose (N=25)
	48 hours	Detectable*	8/8 (100%)		8/8 (100%)		8/8 (100%)
		Median (Min, Max)	561 (131, 1138)		69 (25, 519)		126 (42, 189)
Vaginal TFV-DP (fmol/g) Average LLOQ = 69,100 fmol/g	4 hours	Detectable*	2/9 (22%)	4/12 (33%)	8/8 (100%)	0/12 (0%)	3/8 (38%)
		Median (Min, Max)	33922 (16307, 60366)	30118 (11,907, 76,775)	151001 (45913, 212285)	25260 (7198, 60582)	24137 (11596, 74826)
	24 hours	Detectable*	2/8 (25%)		4/8 (50%)		4/9 (44%)
		Median (Min, Max)	31425 (23666, 59724)		48657 (21320, 136184)		45237 (21454, 67312)
	48 hours	Detectable*	0/8 (0%)		3/8 (38%)		2/8 (25%)
		Median (Min, Max)	37437 (18895, 80566)		44892 (16833, 82135)		39277 (23615, 61238)
Vaginal FTC-TP (fmol/g) Average LLOQ = 63,400 fmol/g	4 hours	Detectable*	9/9 (100%)	12/12 (100%)	8/8 (100%)	12/12 (100%)	8/8 (100%)
		Median (Min, Max)	898085 (327724, 1770766)	691842 (368887, 1785223)	1575008 (591025, 2564975)	619074 (290697, 1410825)	1001657 (364790, 1332966)
	24 hours	Detectable*	8/8 (100%)		8/8 (100%)		9/9 (100%)
		Median (Min, Max)	348473 (130412, 922240)		325210 112622, 677746)		382056 (168810, 1242943)
	48 hours	Detectable*	8/8 (100%)		8/8 (100%)		8/8 (100%)
		Median (Min, Max)	128349 (74259, 383052)		140190 (50328, 273356)		206203 (58565, 277710)
Vaginal dATP (fmol/g) Average LLOQ = 20,976 fmol/g	4 hours	Detectable*	9/9 (100%)	12/12 (100%)	8/8 (100%)	12/12 (100%)	8/8 (100%)
		Median (Min, Max)	264890 (133272, 643247)	228344 (112852, 569636)	234453 (172110, 384025)	280450 (85625, 479768)	190986 (122463, 272623)
	24 hours	Detectable*	8/8 (100%)		8/8 (100%)		9/9 (100%)
		Median (Min, Max)	175394 (68553, 319835)		169929 (42535, 323802)		178269 (52059, 345746)
	48 hours	Detectable*	8/8 (100%)		8/8 (100%)		8/8 (100%)
		Median (Min, Max)	319605 (113041, 471533)		324704 (128222, 384592)		257461 (116391, 379100)
Vaginal dCTP (fmol/g) Average LLOQ = 20,054 fmol/g	4 hours	Detectable*	9/9 (100%)	12/12 (100%)	8/8 (100%)	12/12 (100%)	8/8 (100%)
		Median (Min, Max)	234540 (70216, 598309)	209645 (93331, 538229)	228634 (117689, 392058)	199349 (93240, 507227)	1778819 (100258, 246168)
	24 hours	Detectable*	8/8 (100%)		8/8 (100%)		9/9 (100%)
		Median (Min, Max)	95862 (72945, 211851)		125418 (26737, 184678)		131245 (27657, 278237)
	48 hours	Detectable*	8/8 (100%)		8/8 (100%)		8/8 (100%)
		Median (Min, Max)	251617 (92238, 418928)		202149 (91299, 267816)		201855 (97631, 285260)
Rectal TFV (ng/g) Average LLOQ = 43 ng/g	4 hours	Detectable*	8/8 (100%)		8/8 (100%)		9/9 (100%)
		Median (Min, Max)	297 (47, 1378)		936 (138, 3586)		8293 (556, 46977)
Rectal FTC (ng/g) Average LLOQ = 43 ng/g	4 hours	Detectable*	8/8 (100%)		8/8 (100%)		9/9 (100%)
		Median (Min, Max)	3307 (1558, 5458)		3584 (1575, 18683)		2977 (1200, 6144)
Rectal TFV-DP (fmol/g) Average LLOQ = 96,715 fmol/g	4 hours	Detectable*	4/8 (50%)		6/8 (75%)		9/9 (100%)
		Median (Min, Max)	57075 (31207, 207521)		150,074 (36791, 708600)		2520705 (322820, 19429726)
Rectal FTC-TP (fmol/g) Average LLOQ = 88771 fmol/g	4 hours	Detectable*	5/8 (63%)		4/8 (50%)		8/9 (89%)
		Median (Min, Max)	47505 (28644, 577760)		56075 (39252, 133815)		298530 (48328, 1202347)
Rectal dATP (fmol/g) Average LLOQ = 29349 fmol/g	4 hours	Detectable*	6/8 (75%)		5/8 (63%)		7/9 (78%)
		Median (Min, Max)	27828 (9470, 48758)		17798 (12977, 92368)		32495 (13537, 116973)
Rectal dCTP (fmol/g) Average LLOQ = 30857 fmol/g	4 hours	Detectable*	5/8 (63%)		5/8 (63%)		8/9 (89%)
		Median (Min, Max)	24412 (14506, 53536)		20640 (14630, 44129)		59994 (16799, 194975)

*For time points that had two vaginal or two cervical or four rectal biopsies collected and analyzed, a participant's samples were counted as detectable if at least 1 of the sample type was >BLQ[1]. Average LLOQ is computed for cervical and vaginal samples combined in the SDP and MDP combined, and for rectal samples taken 4 hours post 14th dose in the MDP. Analysis is performed on log-transformed values. Geometric mean is converted to the original scale by taking the anti-log. Note: CV Tissue samples are collected at EVMS at 4 hours, at UPITT at 24 hours, and at Profamilia at 48 hours in the MDP. Rectal tissue collected at 4 hours at UPITT only. Concentrations below LLOQ are imputed as 0.5 * LLOQ.





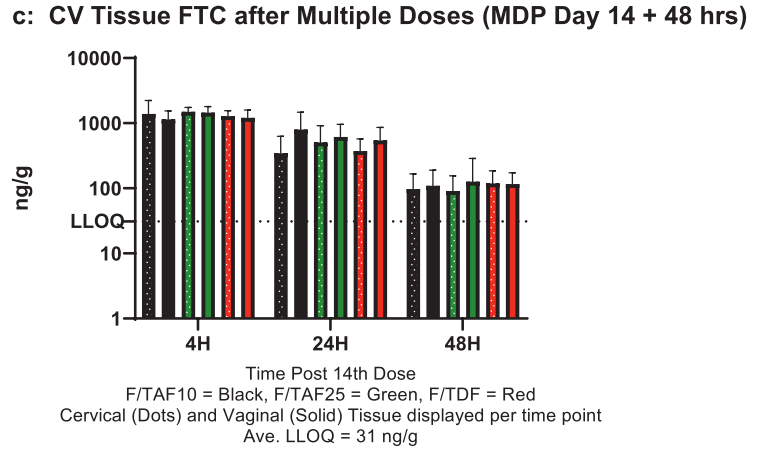
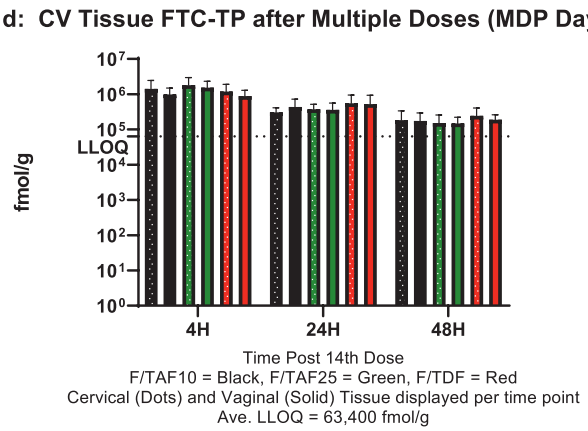
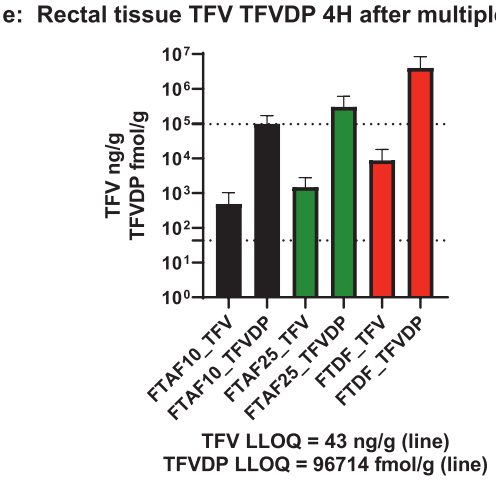


Figure 3a. F/TAF10 = Black, F/TAF25 = Green, F/TDF = Red. Cervical (Dots) and Vaginal (Solid) Tissue displayed per time point. Ave. LLOQ = 31 ng/g
Figure 3b: F/TAF10 = Black, F/TAF25 = Green, F/TDF = Red. Cervical (Dots) and Vaginal (Solid) Tissue displayed per time point. Ave. LLOQ = 69,100 fmol/g
Figure 3c: F/TAF10 = Black, F/TAF25 = Green, F/TDF = Red. Cervical (Dots) and Vaginal (Solid) Tissue displayed per time point. Ave. LLOQ = 31 ng/g
Figure 3d: F/TAF10 = Black, F/TAF25 = Green, F/TDF = Red. Cervical (Dots) and Vaginal (Solid) Tissue displayed per time point. Ave. LLOQ = 63,400 fmol/g
Figure 3e: TFV LLOQ = 43 ng/g (line); TFV-DP LLOQ = 96,714 fmol/g (line)
Figure 3f: FTC LLOQ = 42 ng/g (line); FTCTP LLOQ = 85410 fmol/g (line)





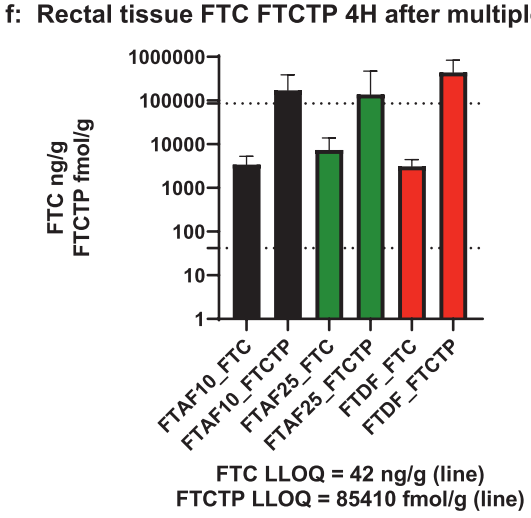


Figure 3a. Continued.

were also low and mostly unquantifiable, but were slightly higher after F/TDF than either F/TAF regimen (figures 4a, 4b). TFV concentrations were higher in rectal fluid after a single dose, with F/TDF achieving the highest level (figure 4c). Cervical, vaginal and rectal fluid concentrations of FTC quickly exceeded 1000 ng/mL after one dose and were similar among all dosing regimens (figures 4a – 4c). At the pre-dose trough on day 7, median and range concentrations of TFV and FTC in cervical, vaginal and rectal fluid overlapped with the day 14 trough concentrations (data not shown). After 14 doses, cervical and vaginal TFV concentrations remained low (figures 4d, 4e) while rectal fluid TFV concentrations were higher, with the highest concentrations seen with F/TDF (figure 4f). After 14 doses, cervical, vaginal and rectal fluid FTC concentrations were high and similar among the 3 study drugs (figures 4d – 4f). Cervical and vaginal concentrations of FTC remained at 1000 ng/mL for approximately 24 hours post the final 14th dose (figures 4e, 4f), while rectal fluid concentrations of FTC remained in this range for up to 72 hours post multiple doses (figure 4f).

Discussion

While there are PrEP efficacy data available in men who have sex with men (MSM) and transgender women from a non-inferiority study supporting a prevention indication for F/TAF25 (the DISCOVER Trial),[15] the multi-compartmental PK data described herein represent the first F/TAF multiple dose comparative data for two doses of F/TAF and the standard dose of F/TDF including analyte values in HIV transmission site tissues of healthy women. These data are important as a Phase III HIV prevention trial in women is in the final planning stages. Although both F/TDF and F/TAF were generally well tolerated during the 14 days of dosing, participants receiving F/TDF had significantly more GI related TEAEs, specifically nausea, compared to F/TAF users. These findings are consistent with previous studies demonstrating efficacy of F/TDF in preventing HIV-1 infection,[3–5] with participants receiving active study drug (F/TDF [3–5] and TDF [3]) reporting GI TEAEs more frequently compared to placebo users, particularly in the first 4 weeks of use.[5] Reports of GI related TEAEs were similar among F/TAF25 versus F/TDF users in the DISCOVER trial, with the incidence of GI related AEs peaking at 4 weeks post PrEP initiation.[15] The frequency of TEAEs during acute (14 days) PrEP use reported here gives insight into the initial experience of

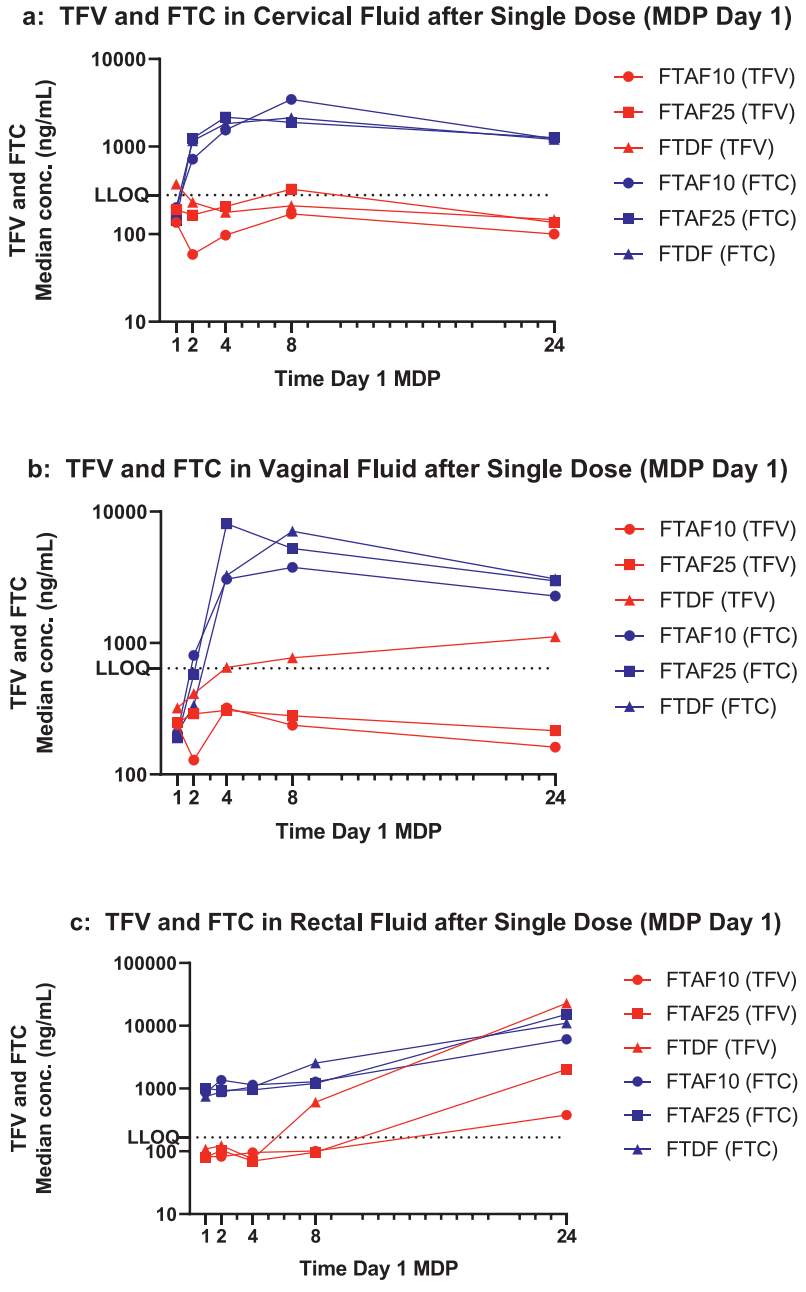


Figure 4a. Red circle = FTAF 10 (TFV); Red square = FTAF 25 (TFV); Red triangle = FTDF (TFV); Blue circle = FTAF 10 (FTC); Blue square = FTAF 25 (FTC); Blue triangle = FTDF (FTC)
Figure 4b: Red circle = FTAF 10 (TFV); Red square = FTAF 25 (TFV); Red triangle = FTDF (TFV); Blue circle = FTAF 10 (FTC); Blue square = FTAF 25 (FTC); Blue triangle = FTDF (FTC)
Figure 4c: Red circle = FTAF 10 (TFV); Red square = FTAF 25 (TFV); Red triangle = FTDF (TFV); Blue circle = FTAF 10 (FTC); Blue square = FTAF 25 (FTC); Blue triangle = FTDF (FTC)
Figure 4d: Red circle = FTAF 10 (TFV); Red square = FTAF 25 (TFV); Red triangle = FTDF (TFV); Blue circle = FTAF 10 (FTC); Blue square = FTAF 25 (FTC); Blue triangle = FTDF (FTC)
Figure 4e: Red circle = FTAF 10 (TFV); Red square = FTAF 25 (TFV); Red triangle = FTDF (TFV); Blue circle = FTAF 10 (FTC); Blue square = FTAF 25 (FTC); Blue triangle = FTDF (FTC)
Figure 4f: Red circle = FTAF 10 (TFV); Red square = FTAF 25 (TFV); Red triangle = FTDF (TFV); Blue circle = FTAF 10 (FTC); Blue square = FTAF 25 (FTC); Blue triangle = FTDF (FTC)

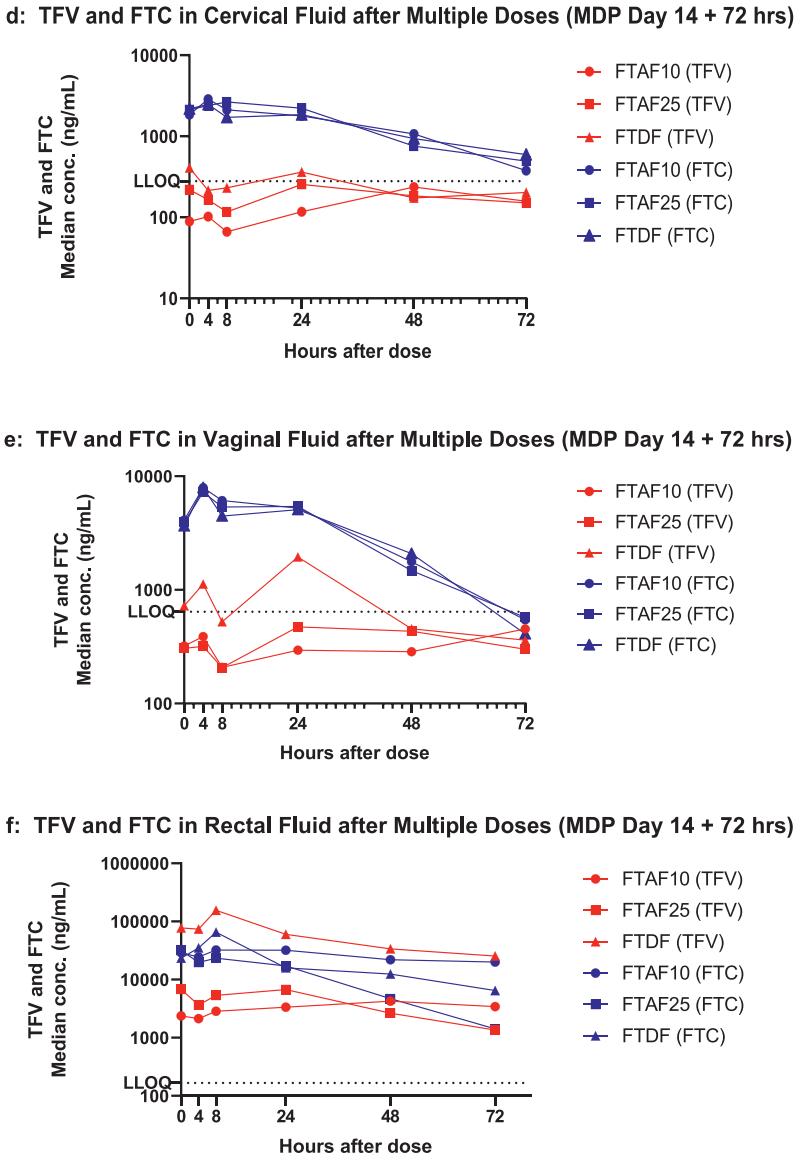


Figure 4a. Continued.

starting PrEP among otherwise healthy women. Although young women face several barriers to initiate and continue oral PrEP, the reduced GI side effects of F/TAF compared to F/TDF may help establishing effective patterns of adherence, particularly in the early stages of PrEP use, when these patterns are being established.

The etiology of TFV-induced GI effects has not been established, but might be linked to the increased circulating plasma TFV with F/TDF compared to F/TAF, which is also thought to be the cause of other side effects on the kidney and bone safety [17–21] (reviewed in [25]). Alternatively, it could be due to increased amounts of TDF/TFV in the lower GI tract due to poorer absorption.[25] In addition, in vitro studies in gastric fluid show that TAF has 6 degradation products while TDF has over 12 degradation products at intestinal pH.[26] Although these GI effects might contribute to acute weight loss, whether they are related in any way to the reported long-term weight loss in women taking TDF-based regimens remains to be ascertained. While data on chronic use of TDF support that GI side effects typically resolve within a few months,[3–5] the experience of nausea in otherwise healthy adolescent girls and young women may have a detrimental impact on adherence, which has historically been problematic in this population.[6–8]

We found differences in both the systemic and mucosal TFV PK profiles of F/TAF versus F/TDF with lower TFV concentrations in plasma and vaginal fluids, and higher concentrations of the active metabolite, TFV-DP, in both PBMCs and CV tissue (at 4 hours post dosing), among F/TAF compared to F/TDF users. This is consistent with data from previous studies among HIV infected participants [16,17] and after one dose of TAF or TDF in healthy women.[27] TAF is less stable in plasma and rapidly penetrates into cells, where it is metabolized to TFV by cathepsin A.[28] In accordance with HIV treatment data, we found approximately 20 to 60 fold lower circulating plasma TFV concentrations with F/TAF versus F/TDF use.[16,17] These lower circulating plasma TFV concentrations observed with TAF-containing regimens used for HIV treatment have been associated with improved measures of long-term renal and bone safety.[17–21]

Consistent with our findings, after oral dosing of F/TDF, TFV-DP concentrations in the rectum are approximately 100x higher than in vaginal tissues.[29] A PK/PD model of PrEP exposure in female genital tract tissue specifically predicts that women need to take 6 – 7 doses of F/TDF per week (which correlates with a concentration of approximately 36 - 42 fmol/10^6 PBMCs [30,31]) to achieve adequate protection against vaginal acquisition of HIV-1.[10] Thus, although there

are no robust, direct PK/seroconversion data in women, as described for MSM,[30] a steady state systemic PrEP benchmark for protection against vaginal acquisition of approximately 40 fmol/10^6 PBMCs of TFV-DP has emerged.[10,31] We found that after one dose of drug, F/TAF users had median TFV-DP PBMC concentrations of almost 200 fmol/10^6 PBMCs while F/TDF users had concentrations below 40 fmol/10^6 PBMCs (figure 2c, table 3). F/TDF users only achieved this benchmark of active metabolite concentration after multiple doses (figure 2d, table 3). Three days after multiple doses, TFV-DP concentrations were significantly higher in the F/TAF25 group, projecting to stay above 40 fmol/M for about 16 days (figure 2d). If protection, at least partially, is conferred by systemic concentrations of active metabolites, this longer tail of F/TAF25 would be more forgiving of missed doses or interrupted pill intake than F/TDF. In our study, treatment regimen was not associated with higher PBMC concentrations of dATP and dCTP (figures 2e, 2f), and therefore these competing nucleotides would have a similar impact on the potency of the three products.[23,24] Thus, our systemic PK data support that F/TAF provides higher TFV-DP levels, which are achieved faster, and may be more forgiving than F/TDF for use in women with suboptimal adherence or with deliberate dosing strategies employing intermittent or on-demand dosing.

There is debate regarding whether orally delivered HIV PrEP products act systemically in PBMCs and at more distant lymphoid tissues and/or act locally at lower genital tract and rectal tissues.[32,33] Animal data support increased systemic potency of TAF, showing that on a per dose basis, TAF led to an increased distribution of TFV in lymphatic tissues compared to TDF.[28] This was confirmed in a cross over study of 13 HIV infected individuals, which showed significantly higher concentrations of TFV-DP in PBMCs and lymphoid tissue with TAF versus TDF use.[33] Our mucosal PK data are consistent with previous studies of F/TDF given to women as a single dose [29,34] or daily for 5 - 6 weeks, showing low to unquantifiable levels of TFV-DP. [31,35] Also consistent with a previous investigation of single dose TAF, TAF was mostly unquantifiable in tissue after a single dose.[27] We believe this is in agreement with the rapid metabolism of the prodrug to its intracellular active metabolite. As previously demonstrated,[29] we found high mucosal concentrations of FTC and FTC-TP in the CV tract, with no seeming advantage of F/TAF over F/TDF regimens, or vice versa, for these analytes. CV tissue concentrations of TFV were lower 4 hours after the multiple dose phase for F/TAF versus F/TDF (figure 3a, table 4), but F/TAF25 users had 3 to 6 fold higher concentrations of the active metabolite, TFV-DP, in cervical and vaginal tissue, compared to F/TDF users (figure 3b, table 4). This is likely due to TAF lymphotropic properties.[22,33] Among pooled cervical and vaginal tissue samples collected at the EVMS site (i.e., 4 hours after the 14^{th} daily dose), 88% from the F/TAF25 treatment arm exhibited quantifiable TFV-DP versus only 25% from the F/TDF treatment arm (table 4). In those samples with quantifiable TFV-DP, median TFV-DP concentrations in cervical and vaginal tissue were 4.5 fold higher at 4 hours after 14 doses in F/TAF25 users compared to F/TDF users (pooled CV tissue medians 138,649 fmol/g versus 30,829 fmol/g respectively).

Among the 8 women dosed at the EVMS site during the multidose phase with F/TAF25, 63% and 88% exhibited cervical and vaginal exposure above published TFV-DP protective target exposure when accounting for their dATP concentration (EC90 TFVdp:dATP = 0.29) while only 14% and 13% respectively were above this threshold in the F/TDF arm.[10] Similarly, when these tissue TFV-DP concentrations were extrapolated to fmol/10^6 female genital tract (FGT) cells (assuming 280,000 cells/mg, unpublished data), 75% and 100% of cervical and vaginal concentrations were above TFV-DP target exposure (TFV-DP EC90=158 fmol/million CD4+ cells; [10]) at 4 hours after the final 14^{th} dose of F/TAF25, while only 29% and 38% respectively reached this level with F/TDF. Based on the long intracellular half-life of TFV-DP in cervical and vaginal tissues (34-53 hours estimated in cervical and vaginal tissue homogenates [34]) the large decrease in the proportion of quantifiable cervical and vaginal tissues collected at 24 hours post dose (28% of pooled evaluable samples, table 4) at the UPITT site, the only site that evaluated this time point, among women taking F/TAF25, was unexpected. Assuming the more conservative half-life estimate of 34 hours, we would have only expected a 1.5-fold decrease in concentrations over this time frame. Given that most concentrations (all but two tissues) observed at 4 hours following F/TAF25 were >1.5-fold above the LLOQ, we would have expected a similar proportion of quantifiable concentrations at 24 hours. The unexpectedly rapid rate of TFV-DP clearance in our study may be an artifact of study design. For logistical and regulatory reasons, we did not randomize tissue collection times and the EVMS site collected tissue at 4 hours post dose, the UPITT site at 24 hours and the Profamilia site at 48 hours.

Although all three sites flash froze the cervical and vaginal tissue samples immediately after collection in the clinic, between site differences in tissue collection techniques, storage and shipping may have confounded these concentration versus time data. Assuming an expected rate of TFV-DP clearance ($T_{1/2} \geq 34$ hours) and based on concentrations observed at 4 hours post dose at least 63% of FGT tissues collected from women dosed with the F/TAF25 group would have been expected to be above EC90 targets at 24 hours post dose. Importantly, TFV-DP CV tissue levels and percentage of samples with quantifiable analyte were higher in the F/TAF25 arm than in the F/TDF arm across all the time points analyzed (figure 3b).

Given the lower levels of TFV-DP in the CV compartment, the assumed CV mucosal action of F/TDF and F/TAF may likely be more related to the FTC component rather than the TDF/TFV component, whose action may be more important at the systemic and rectal compartment levels. [3,4] In the MTN 001 study, 81% of vaginal tissue samples of women taking F/TDF had TFV-DP concentrations below the LLOQ.[35] Similarly, we found that after 14 daily doses of F/TDF, between 54% and 100% of participants had unquantifiable concentrations of cervical and vaginal tissue TFV-DP between 4 and 48 hours post 14^{th} dose (table 4), but most (89% - 100%) had quantifiable, high concentrations of FTC-TP at all post 14^{th} dose time points. Similarly, in the daily dosing arm in HPTN 066, TFV-DP concentrations in vaginal tissue homogenate were mostly unquantifiable with a maximum of 41 fmol/mg after 5 weeks of daily, directly observed, dosing.[31] Importantly, all cervical and vaginal tissue samples collected after 14 doses of F/TAF25 at 4 hours (EVMS) and 24 hours (UPITT) and 97% of those collected at 48 hours (Profamilia) exhibited high FTC (figure 3c) and FTC-TP (figure 3d) concentrations and an FTC-TP exposure above published modeled efficacy targets (i.e. FTC-TP:dCTP = 0.07 and FTC-TP = 10^3fmol/million cells [10]).

Although the female genital tract serves as the first site of entry of HIV infection during vaginal intercourse,[36,37] the relative importance of PK exposure within CV tissues and the correlation with efficacy is currently unknown, with no data directly linking seroconversions to tissue PK concentrations in women. We quantified FTC-TP in all tissues in this study, and found similarly high concentrations of FTC and FTC-TP with F/TAF regimens compared to F/TDF. While FTC-TP was not measured in the MTN 001 study,[35] we found higher concentrations of FTC in vaginal tissue compared to the HPTN 066 study, but similar FTC CV fluid concentrations with all oral regimens.[31] By day 14, we found FTC-TP CV tissue concentrations that were 5 – 10 times higher than those found in macaques after single dose of TDF (20 mg/kg) and FTC (22 mg/kg).[38,39] In these macaque studies, F/TDF conferred 100% protection to animals without coexisting STIs [39] and 67% protection to animals co-infected with STIs.[38] PBMC concentrations of TFV-DP and FTC-TP were similar among infected versus uninfected animals, but the infected animals who received TDF and FTC had reduced genital virus shedding compared to infected, control macaques.[38] The authors stated that this provided evidence for mucosal drug penetration and local anti-

viral activity.[38] Regarding the contribution of FTC to prevention of mucosal HIV acquisition, in another study by the same group, the protection afforded by TAF alone in macaques was approximately 57.8%,[40] with low levels of TAF detected in tissue, consistent with our findings and those of our colleagues [27] and further supporting the role of mucosal antiretroviral (ARV) contribution to protection. The protection afforded by the F/TAF combination, in turn, was 91%, supporting the role of FTC in protecting the animals from vaginal infection.

Thus, based on the CV mucosal PK profiles, collectively our data support that while both F/TAF and F/TDF likely offer similar mucosal protection, F/TAF25 not only increases tissue TFV-DP, but likely offers enhanced/more prolonged systemic protection, as evidenced by higher TFV-DP concentrations in PBMCs. CV and rectal fluid concentrations of FTC were similarly high among F/TAF and F/TDF users, within HIV inhibitory range [10] and consistent with tissue data, supporting our hypothesis that although both ARVs may contribute to protection against HIV mucosal infection, FTC may be more important at the CV mucosal level, while TFV may contribute more to systemic protection. In combination and at concentrations achieved by consistent adherence to daily intake, several layers of protection are therefore in place while taking either combination of FTC with TAF or TDF.

We also observed lower concentrations of TFV in rectal tissues following 14 days of treatment with F/TAF than with F/TDF (table 4) and approximately 16.8-fold higher TFV-DP concentrations following treatment with F/TDF than with F/TAF25 (table 4). The fact that TDF is not absorbed as well orally as TAF [41] may explain these differences in rectal concentrations and may also explain why more GI TEAEs are observed with F/TDF compared to F/TAF. Since TDF is available to transit through the stomach and intestines, it may accumulate more in the GI tract, which has a high density of immune cell, and locally convert to TFV-DP. In other words, TFV-DP in rectal tissue may come from local as well as systemic sources. As in CV tissue exposure, the full implication of rectal mucosal PK exposure is unknown. While local tissue concentrations may play a role in HIV-1 acquisition during anal intercourse, it is also likely that systemic concentrations may be critically important to prevent infection in lymph nodes and other organs.[42] In the DISCOVER trial, both F/TAF and F/TDF were highly effective, and F/TAF was non-inferior to F/TDF, at preventing HIV infection through rectal transmission.[15]

Limitations of this study include short dosing duration (women received two weeks of study products), inability to obtain multiple tissue samples from each participant (due to safety and regulatory constraints), and the lack of randomization to collection time points between the clinical sites. As such our tissue PK estimates (AUC and Cmax) should be interpreted with caution. Although this study evaluated two F/TAF formulations that contained two different doses of TAF, F/TAF containing 25 mg of TAF, currently marketed as Descovy®, is the only one approved by US FDA for HIV prevention. Compliance with study drug was assured in the single dose phase as DOT was utilized. In the multi-dose phase, participants ingested doses 1, 2, 7 and 14 in the clinic, but we relied on their report and returned pills for compliance with home dosing. We believe that study participants were not in contact with each other during the study, as we did not enroll groups of peers, but the fact that the F/TDF and F/TAF pills look different and that study participants and investigators were not blinded to study product is a potential limitation of this study. A double dummy method design would have been stronger, but this was not feasible. We restricted enrollment to women with a BMI of body mass index ≥ 18 and < 35 kg/m^2, as F/TDF is approved for use as PrEP in high risk individuals weighing over 35 kg. By including individuals with BMIs ranging from normal to obese, this study gives the PK profiles of a wide range of healthy women. Although this study was conducted among healthy women in the US and Dominican Republic, our PK profiles of F/TDF are consistent with previous studies. [10,23,29,31]

In conclusion, F/TAF appears to be safe and well-tolerated in this population of healthy women. In the systemic compartment, F/TAF25 showed higher levels of TFV-DP and similar levels of FTC-TP in PBMCs than F/TDF. In the rectal compartment, F/TAF25 showed lower levels than F/TDF, but DISCOVER trial data clearly demonstrated that F/TAF25 was non-inferior and possible slightly better than F/TDF in preventing rectal acquisition.[15] Finally, in the CV tract, FTC and FTC-TP concentrations are high and similar for both drug combinations with F/TAF showing higher levels of TFV-DP than F/TDF four hours after last dose. F/TDF has been proven effective in preventing vaginal acquisition of HIV [3,4]. Based on this comparative analysis and taken into consideration multi-compartmental PK and previous efficacy data of the drugs, we speculate that, given adequate adherence, F/TAF25 should be protective against vaginal acquisition of HIV to a similar or even greater degree than F/TDF. Altogether these findings support F/TAF as a potentially safer, more potent and forgiving HIV PrEP ARV combination for women.

Declaration of Competing Interest

Dr Schwartz received funding from USAID to support this study. Dr. McCallister and Dr. Rooney are shareholders in Gilead Sciences. Dr. McCallister was a Gilead employee at the time of the study and Dr. Rooney is currently a Gilead Sciences employee. Dr. Chen reports grants from Medicines360, grants from Sebela, personal fees from Merck, outside the submitted work. Dr. McGowan reports grants from CONRAD, during the conduct of the study; other from Orion Biotechnology, outside the submitted work. All the other authors report no conflicts.

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Data Sharing Statement

Summary data are available upon request of the corresponding author.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.eclinm.2021.100893.

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