A Translational Pharmacology Approach to Predicting HIV Pre-Exposure Prophylaxis Outcomes in Men and Women Using Tenofovir Disoproxil Fumarate ± Emtricitabine

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Abstract

Background: A novel translational pharmacology investigation was conducted by combining an in vitro efficacy target with mucosal tissue pharmacokinetic data and mathematical modeling to determine the number of doses required for effective HIV pre-exposure prophylaxis (PrEP).

Methods: A pharmacokinetic/pharmacodynamic (PK/PD) model was developed by measuring mucosal tissue concentrations of tenofovir, emtricitabine, their active metabolites [tenofovir-diphosphate (TFVdp), emtricitabine-triphosphate (FTCtp)], and competing endogenous nucleotides (dATP, dCTP) in 47 healthy women. TZM-bl and CD4⁺ cells were used to identify EC₉₀ ratios of TFVdp:dATP and FTCtp:dCTP (alone and in combination) for protection against HIV. Monte-Carlo simulations were then performed to identify minimally effective dosing strategies to protect lower female genital tract (FGT) and colorectal tissues.

Results: Colorectal TFVdp was 10 times higher than FGT while endogenous nucleotides were 7-11 times lower. Our model predicted ≥98% of the population achieve protective mucosal tissue exposure by the third daily dose of tenofovir disoproxil fumarate + emtricitabine. However, a minimum adherence of 6/7 (85%) doses/week was required to protect FGT tissue from HIV, while 2/7 (28%) doses/week was required for colorectal tissue.

Conclusions: This model is predictive of recent PrEP trial results where 2-3 doses/week was 75-90% effective in men but ineffective in women. These data provide a novel approach for future PrEP investigations that can optimize clinical trial dosing strategies.
Background:

A fixed dose, combination tablet of tenofovir disoproxil fumarate (TDF) 300mg and emtricitabine (FTC) 200mg received FDA approval for HIV pre-exposure prophylaxis (PrEP) on the basis of two trials. Partners PrEP demonstrated 75% efficacy among heterosexual serodiscordant couples[1] and iPrEx demonstrated 44% efficacy among men who have sex with men (MSM) and transgender women.[2] However, two subsequent trials investigating daily TDF±FTC in high risk women (FEM-PrEP and VOICE) failed to demonstrate efficacy.[3, 4] Analysis of plasma drug concentrations from these studies revealed only 24-30% of these women exhibited evidence of recent product use. Yet in a cohort of male participants from the successful iPrEx trial, only 28% had evidence of recent product use.[2] Subsequent analysis revealed drug exposure consistent with 2-3 doses/week achieved 75-90% protection in MSM.[5, 6] These findings pose an important question: Why are adherence requirements for effective PrEP less strict for men?

We recently demonstrated exposure of TDF’s active metabolite (tenofovir-diphosphate; TFVdp) is 100-fold higher in colorectal tissue relative to female genital tract (FGT) tissue.[7] This may partially explain different adherence requirements in men and women. Another potential factor is the concentration of host cells’ endogenous 2’deoxynucleotides analogs, deoxyadenosine triphosphate (dATP) and deoxycytidine triphosphate (dCTP). The active intracellular phosphorylated metabolites of TFV and FTC (TFVdp and emtricitabine-triphosphate (FTCtp), compete with dATP and dCTP for incorporation into the proviral DNA strand to terminate chain elongation.[8] Since dATP and dCTP can be modulated by proinflammatory signaling molecules[9, 10], and mucosal tissues may have environments with altered inflammatory states, it is possible that endogenous nucleotides differ between cervical,
vaginal and colorectal tissue, and contribute to altered antiviral efficacy of TFVdp and FTCtp.

To investigate mechanisms of efficacy within FGT and colorectal tissues, we used a quantitative systems pharmacology approach to predict the probability of protection against HIV with varying patterns of adherence to TDF, FTC, and TDF+FTC.

Methods:

Study Design

We conducted a phase I, single dose, pharmacokinetic investigation of TDF and FTC at 50, 100, or 200% of the treatment dose (NCT01330199). Blood was collected at baseline and over 48hrs and each participant provided one cervical, vaginal, and colorectal tissue sample at 6, 12, 24, or 48hrs post-dose. Drug concentration were quantified in plasma, peripheral blood mononuclear cells (PBMCs), and tissues. The study was conducted in accordance with Good Clinical Practice procedures and all participants provided written informed consent before study entry. Details about the clinical trial, sample processing and analytical methods are provided in the Supplementary Methods.

In vitro Concentration vs Response

TZM-bl reporter cells (NIH AIDS Reagent Program, Germantown, MD, USA)[11, 12] and human CD4+ cells were used to determine HIV-1 infection in the presence of tenofovir (TFV) and FTC. 100,000 TZM-bl cells were plated in 6-well, flat bottom culture plates in 2ml of DMEM medium supplemented with 10% fetal bovine serum (FBS) and antibiotics to match the experimental conditions for drug quantification where >100,000 cells were needed. Cells were incubated in TFV (0.05-35μM), FTC (0.03-30μM), or their combination (NIH AIDS Reagent Program) for 24hrs at 37°C. 5μl of HIV-1JR-CSF (NIH AIDS Reagent Program; MOI=0.006) and
5μl DEAE-dextran were added to each well. This HIV-1JR-CSF dose was selected to ensure infection, as measured by luminescence, was within linear range. After 48hrs, cells were lysed with 500μl of GloLysis Buffer (Promega, Madison, WI, USA), and relative light units (RLU) were measured using a Luciferase Assay System kit (Promega) on a Veritas™ Microplate Luminometer (Turner Biosystems, Sunnyvale, CA, USA). Infectivity was normalized to infection in the absence of drug and reported as %inhibition.

PBMCs isolated from buffy coats obtained from New York Blood Center (Long Island City, NY, USA) were sorted for CD4+ lymphocytes using an EasySep™ negative selection kit (STEMCELL Technologies Inc, BC, Canada). Typical cell yields using this kit demonstrate ~96% purity and >90% viability. CD4+ cells were stimulated for 48hrs in RPMI media supplemented with 10%FBS, antibiotics, phytohaemagglutinin (PHA, 5μg/ml), and interleukin-2 (10units/ml). 1x10^6 CD4+ cell/ml were resuspended and incubated in PHA-free, RPMI media as prepared above with either TFV (0.3-10μM) or FTC (0.03-30μM) for 24hrs. Pseudotyped virus was generated using NL4-3.Luc.R-.E-backbone (NIH AIDS Reagent Program)[13, 14] and transfected with env expression plasmid from HIV-1JR-CSF using X-tremeGENE HP (Roche Life Science, Indianapolis, IN, USA). Virions were concentrated 10-fold using Lenti-X Concentrator (Clontech Laboratories, Inc., Mountain View, CA, USA). Cells were incubated for 48hrs in 25μl of pseudotyped virus (MOI=1) then lysed with 100μl of GloLysis Buffer. Infection was reported as described above.

Cellular active metabolite:endogenous nucleotide concentration ratios were quantified with an identical set of uninfected TZM-bl or CD4+ cells. Cells were harvested at the time of HIV-1 challenge (24 after incubation in drug), washed, counted, lysed in 70%methanol/water, and stored at -80°C for TFVdp, FTCtp, dATP, and dCTP quantification.
Pharmacokinetic Analysis

AUC\textsubscript{0-48hr} was calculated individually using the linear up/log down trapezoidal rule for plasma and PBMCs with WinNonlin\textregistered v6.3 (Pharsight Corporation, CA, USA). AUC\textsubscript{0-48hr} was calculated using sparsely sampled data and the linear trapezoid rule for tissue. Concentrations below the limit of detection or quantification were imputed as 10% or 50% of the lower limit of quantification, respectively.

Monte-Carlo Simulations were performed with a population pharmacokinetic model built using the clinical trial concentration data and NONMEM\textsuperscript{v7.4}, ICON plc. Simulations were performed for the first 10 doses and at steady state with dosing intervals representing 1-7 doses/week. Time to steady-state was defined as the first dose at which the 24hr concentration (C\textsubscript{trough}) was within 10% of simulated steady-state C\textsubscript{trough}.

Statistical Analysis

Dose proportionality was assessed in tissues across a 4-fold dosing range to assist with pharmacokinetic modeling using the Holder method\cite{15} and declared if the 90% confidence interval (CI) around the regression line slope (\( \hat{\beta}_1 \)) for log transformed dose vs concentration fit within 0.64-1.36. This analysis was conducted using R\textsuperscript{v2.14}.\cite{16} Assuming %coefficient of variation (CV)\( \leq \)45\%, 8 women/dosing level provided 83\% power to declare dose proportionality. Between-tissue comparisons of endogenous nucleotides were made using one-way ANOVA with Bonferroni correction. Descriptive and comparative statistics were performed with SAS\textsuperscript{v9.3} (SAS Institute Inc., Cary, NC, USA).

For cellular data, exposure vs response was described using Emax regression (Eq 1, \( E_0 \) fixed to 0, \( E_{max} \) fixed to 1, \( E=\text{Response}, \text{MR= molar ratio} \)) for active metabolite:endogenous
nucleotide vs fraction inhibition of HIV-1 infection. Negative values were fixed at 0. Synergy was assessed with a pharmacodynamic interaction model (ψ, Eq 2)[17] for TFVdp:dATP and FTCtp:dCTP in TZM-bl cells by fixing $EC_{50}$ and Hill slope (H) parameters from Eq 1. A 3-D goodness of fit plot was generated and visually inspected to evaluate model predictions for bias. The final fitted parameters ($H_{TFV}$, $H_{FTC}$, $EC_{50,TFV}$, $EC_{50,FTC}$, $\psi$) were used to predict %inhibition from simulated concentrations, with an efficacy target of 90%.

(Eq 1) $E = E_0 + \frac{MR^H \times E_{\text{Max}}}{MR^H + EC_{50}^H}$

(Eq 2) $E = \frac{\left(\frac{TFV}{\psi \times EC_{50,TFV}}\right)^{H_{TFV}}}{1 + \left(\frac{TFV}{\psi \times EC_{50,TFV}}\right)^{H_{TFV}}} + \frac{\left(\frac{FTC}{\psi \times EC_{50,FTC}}\right)^{H_{FTC}}}{1 + \left(\frac{FTC}{\psi \times EC_{50,FTC}}\right)^{H_{FTC}}} + \frac{\left(\frac{TFV}{\psi \times EC_{50,TFV}}\right)^{H_{TFV}}}{1 + \left(\frac{TFV}{\psi \times EC_{50,TFV}}\right)^{H_{TFV}}} \times \frac{\left(\frac{FTC}{\psi \times EC_{50,FTC}}\right)^{H_{FTC}}}{1 + \left(\frac{FTC}{\psi \times EC_{50,FTC}}\right)^{H_{FTC}}}$

**Results:**

**Participant Demographics and Safety**

Between April 2012 and August 2013, 49 healthy female volunteers gave consent for the clinical trial. Participant demographics are summarized in Table 1 and stratified by treatment arm. Eight participants were enrolled into each dosing group except TDF 300mg where one participant was unable to provide samples and was withdrawn and replaced. Samples from one participant dosed with FTC 200mg were not analyzed because of improper storage. Single doses
of TDF and FTC up to 200% of the licensed treatment dose were well tolerated with no adverse events above Grade 1 (Table 1).

Drug Concentrations and Dose Proportionality

Figure 1 depicts area under the concentration-time curve vs dose for extracellular concentrations and intracellular metabolite concentrations in plasma, PBMCs, and tissues. Table 2 summarizes dose proportionality statistics. Plasma FTC increased dose proportionally. Plasma TFV did not meet dose proportionality criteria but exhibited dose linearity. PBMC concentrations of TFVdp, but not FTCtp, increased dose proportionally. Since no notable difference was observed between cervical and vaginal tissue concentrations, they were averaged for subsequent analyses. A single composite tissue AUC$_{0-48hr}$ was calculated for each dosing group and tissue type. Only FGT emtricitabine concentrations increased dose proportionally.

Median dose adjusted AUC$_{0-48hr}$ for TFV and TFVdp were 10-45 times higher in colorectal tissue (38.5µg*hr*g$^{-1}$ and 2046.5pmol*hr*g$^{-1}$, respectively) compared with FGT tissue (0.83µg*hr*g$^{-1}$ and, 188pmol*hr*g$^{-1}$, respectively). Although median dose adjusted FTC AUC$_{0-48hr}$ was higher in colorectal tissue (222.3µg*hr*g$^{-1}$) than FGT tissue (17.6µg*hr*g$^{-1}$), FTCtp was 140 times higher in FGT tissue (15094.3pmol*hr*g$^{-1}$) than colorectal tissue (108.2pmol*hr*g$^{-1}$). Concentration vs time profiles for the blood and tissues are provided in Supplementary Figure 1 and 2, respectively.

Human Mucosal Tissue Endogenous Nucleotides

Although dATP concentrations were similar in vaginal and cervical tissues (Figure 2A), dCTP concentrations were 50% lower in vaginal vs cervical tissue (Figure 2B). Colorectal tissue contained lower endogenous nucleotide pools compared to the FGT: dATP concentrations were
5 times lower (p<0.05), and dCTP concentrations were 5-11 times lower (p<0.05). In all tissues, dATP and dCTP concentrations were highly correlated (cervical Pearson’s r=0.84, vaginal Pearson’s r=0.92, colorectal Pearson’s r= 0.73; p<0.0001). No differences in PBMC or tissue dATP/dCTP concentrations were noted between treatment arms, dosing groups, or sample collection time. In the female genital and colorectal tissues TFVdp:dATP ratios ranged from 0.00074 to 0.14 and 0.0014 to 5.9, respectively; and FTCtp:dCTP ranged from 0.025 to 17 and 0.016 to 1.4, respectively.

**Pharmacokinetic Modeling**

An 8 compartment model with 7 gastrointestinal transit compartments (Supplementary Figure 3) best fit the clinical trial concentration data. Model parameters (Supplementary Table 1) and goodness of fit have been previously reported.[18] A 1000-subject Monte-Carlo simulation was performed to simulate TFVdp:dATP and FTCtp:dCTP in FGT and colorectal tissue over the dosing interval for the first 10 daily doses and under steady-state conditions for each drug alone and in combination. TFVdp reached steady-state concentrations by dose 3 in FGT and dose 9 in colorectal tissue. FTCtp reached steady-state by dose 2 in FGT and dose 6 in colorectal tissue.

**In Vitro Concentration vs Response**

We investigated targets for protective efficacy against HIV infection in both a cell line and primary human cells. Figure 3 illustrates the best fit Emax parameters for active metabolite:endogenous nucleotide ratios and protection from HIV infection. For TFVdp:dATP the estimated EC\textsubscript{90} ratio was 0.086 (TZM-bl cells) and 0.29 (CD4\textsuperscript{+} cells). For FTCtp:dCTP the estimated EC\textsubscript{90} ratio was 0.27 (TZM-bl cells) and 0.07 (CD4\textsuperscript{+} cells). Emax model parameters for culture medium drug concentration and intracellular active metabolite vs %inhibition are
provided in Supplementary Table 2. TFVdp:dATP and FTCtp:dCTP demonstrated synergy in TZM-bl cells \( (\mu(SE)=0.63(0.074); p<0.001 \) where \( \mu <1 \) indicates synergy]. A synergistic interaction was also noted between TFV and FTC in CD4+ cells (data not shown).

**Pharmacokinetic/Pharmacodynamic Simulations**

EC\_90 ratios derived from the more biologically relevant activated CD4+ Emax model was used as target efficacy exposure for monotherapy simulations with TDF or FTC. The \( \mu=0.63 \) modeled a surface to describe all possible EC\_90 combination ratios when using TDF+FTC. To quantify the difference in predictions produced by the two pharmacodynamic models, we simulated 1000 clinically relevant TFVdp:dATP and FTCtp:dCTP ratios. The predicted proportion of the population achieving target exposure differed by \( \leq 2\% \) between the two models.

In colorectal tissue the maximal proportion of the population (100%) achieved target exposure for efficacy after 3 daily doses of the fixed dose combination (Figure 4A). In FGT tissue the maximal proportion of the population (99%) achieved target exposure over the entire dosing interval after 3 daily doses of the fixed dose combination (Figure 4C). In colorectal tissue, dosing twice/week with the fixed dose combination achieved target exposure in >95% of the population (in FGT, this dosing achieved target exposure in 65%; Figure 4B,D).

To predict pericoital dosing effectiveness, we simulated colorectal tissue exposure achieved by the Ipergay dosing regimen (#2 doses 2-24hrs before coitus, #1 dose 24hrs after coitus, and #1 dose 48hrs after coitus) for TDF alone and with FTC. When administered 2 and 24hrs before coitus, TDF+FTC achieved target exposure (at the time of coitus) in approximately 81% and 98% of the population, respectively (Figure 5A,B). Target exposure was sustained out to 240hrs after coitus. In FGT, when administered 2 and 24hrs before coitus, TDF+FTC achieved
target exposure at the time of coitus in 98% and 100% of the population, respectively (Figure 5C,D). The concentrations in the FGT were short-lived compared to colorectal tissue: <85% of the population had target exposure at 120hrs following coitus.

Discussion:

Phase III clinical trials evaluating daily oral TDF±FTC for HIV PrEP have demonstrated mixed results in heterosexual women.[1, 3, 4, 19] To understand this, we generated comprehensive pharmacokinetic and biological data in vulnerable mucosal tissues and evaluated effective drug exposure with different dosing strategies. To our knowledge, this is the first biologically complete model to predict effective PrEP dosing. This analysis generated several important findings: 1) Confirmation that TFV and FTC have different tissue distribution characteristics where TFV favors colorectal tissue and FTC favors FGT tissue; 2) Characterization of the intracellular active metabolite:endogenous nucleotide ratio as an accurate pharmacodynamic target for NRTI's, whereby endogenous nucleotides were found in higher concentration in FGT tissue, and 3) Evidence that this PK/PD approach can be used to develop optimized TDF±FTC PrEP dosing strategies and predict clinical trial outcomes with different adherence patterns prior to the trials being conducted.

Previously, Patterson et. al. described 100-fold higher TFVdp concentrations in colorectal compared to FGT tissue with a single TDF+FTC dose.[7] Herein, we investigated 50% to 200% of the licensed treatment doses to develop a robust pharmacokinetic model of TFVdp and FTCtp distribution. Our findings are consistent with this previous report: TFVdp concentrations overall were 10 times higher in colorectal tissue while FTCtp concentrations were 140 times higher in FGT tissue. Between-tissue differences in drug transport systems may explain differential
concentrations; yet blood flow to rectal and vaginal mucosa is similar.[20] Drug transport by lymph fluid into tissues has not been fully characterized. The lipid rich lymph fluid may not favorably transport hydrophilic compounds like TFV and FTC.[21, 22] However the leaky lymphatic endothelium could provide an alternative mode of absorption in colorectal tissue,[23] possibly explaining the delayed peak we observed in this tissue. We also found that dATP and dCTP concentrations were 7 and 11 times lower in colorectal tissue compared with FGT tissue, respectively. Therefore, not only were TFVdp concentrations much higher in colorectal tissue, but we found that the colorectal intracellular environment favors TFV’s prophylactic activity.

A significant limitation in determining PrEP concentration targets for efficacy is the inability to easily measure the PK/PD relationship in humans. HIV prevention models rely on extrapolating data from cell culture systems, human tissue explants, or animal studies, each of which has limitations.[24, 25] Primate models utilize SIV or SHIV that may not mimic HIV’s response to antiretrovirals;[26] antiretroviral exposure in humanized mouse models may not match humans;[26] and ex vivo tissue explants’ endogenous nucleotides deteriorate by ≥80% within 24hrs of isolation.[27] Although a homogenous cell population fails to represent the 3-dimensional architecture and diverse microanatomy involved in transmission events, these models can be infected with HIV, exposed to clinically relevant antiretroviral concentrations, and sustain endogenous nucleotide pools.[28] Since active metabolite concentrations in isolated mononuclear rectal cells strongly correlate with tissue homogenate concentrations,[29], we believe using isolated target cells to generate concentration-effect relationships for NRTI prevention strategies is reasonable.

A number of previous studies have highlighted the importance of endogenous nucleotides in NRTI efficacy.[30-32] Therefore, we postulated significant differences in tissue endogenous
nucleotide concentrations may affect the PK/PD relationships for HIV prevention. Using primary (human CD4+ cells) and immortalized (TZM-bl cells) culture systems with a clinically relevant HIV-1 challenge, we identified efficacy targets for TFVdp and FTCtp relative to their competing endogenous nucleotides (dATP and dCTP). Differences in the concentration-response relationship between in vitro systems were noted. However, normalizing active metabolite to endogenous nucleotide concentrations reduced the difference between TZM-bl and CD4+ EC90 ratio values by 70% (Supplement Table 2), and resulted in a difference of <2% in clinical predictions between these model systems, highlighting the importance of the endogenous nucleotide exposure.

Combining the mathematical pharmacokinetic mucosal tissue model with the cellular target ratios, we determined the maximal proportion of the population achieved effective FGT and colorectal concentrations after 3 doses of TDF+FTC. Our data are consistent with the Cell-PrEP study.[33] These data differ from the CDC guidelines, which are built on extrapolations from PBMC data[34]. However, since they are based on tissue pharmacokinetics, we believe they better inform clinical utility. Importantly, we found 2 doses/week of TDF±FTC achieved effective colorectal concentrations in >95% of the population, which emulate iPrEx-OLE results (90% protection with 2-3 TDF+FTC doses/week).[6]

To predict colorectal protection with pericoital oral dosing, we evaluated the Ipergay dosing regimen.[35] When a double dose of TDF+FTC is taken 24hrs before coitus, and 2 doses are taken over 48hrs following coitus, we demonstrate >95% of the population achieves effective concentrations in colorectal tissue at the time of, and for ≥240hrs after, coitus. When administered 2hrs before coitus, approximately 17% of the population are below the target for the first 4hrs after coitus. However, this is likely an inconsequential delay, since it takes 4hrs for
reverse transcription to occur after HIV binds to cellular membranes[36]. These findings reasonably emulate clinical trial data whereby this dosing regimen demonstrated 86% efficacy in MSM.[35]

Our data show daily TDF+FTC is required for 100% of the population to be protected from HIV infection within the FGT. Our efficacy predictions are in agreement with the Partners PrEP trial, which demonstrated 92% risk reduction (95% CI=19-99%) when women taking TDF+FTC for PrEP had plasma drug concentrations consistent with very high adherence.[37] We also simulated the Ipergay regimen for the FGT. Using this approach, >95% of the population achieve target exposure in FGT tissue at the time of, and for 72hrs after, coitus. However, the effect is comparatively short lived, as by 120hrs following coitus <85% are still at target. Administering TDF+FTC 2 or 24hrs before coitus did not change the proportion achieving target exposure in the FGT tissue across this time frame. To match the percent of the population achieving target exposure in colorectal tissue with Ipergay dosing, women would need to take 9 daily doses of TDF+FTC following coitus. These data suggest this may not be a strongly protective regimen for women vaginally exposed to HIV and are consistent with recent preliminary reports from clinical trials where more women using intermittent dosing acquired HIV compared to those using daily dosing.[38]

Our approach has certain limitations. First, our in vitro methods necessitated the use of PHA/IL-2 stimulation which may increase the phosphorylation of NRTIs and endogenous nucleotides in CD4+ cells.[9] However, direct measurement of active metabolites and normalization to endogenous nucleotides helped minimize confounding. Although the exposure-response relationship was defined under conditions of static drug exposure, given the long intracellular half-lives of TFVdp and FTCtp (~48 and 39 hours, respectively)[39, 40] marked
fluctuation of active metabolite concentrations in tissues is not expected, and has not been identified. The concentration data used to build the pharmacokinetic model was obtained over 48 hours. However, there is precedent for making steady state drug exposure predictions from similarly developed models.[41] Further, our predicted steady state plasma concentrations were consistent with literature values, and our predicted time to steady state in colorectal tissue (9 days for TFVdp) was consistent with previous reports.[33] The use of tissue biopsy homogenates to measure mucosal tissue drug concentrations assumes uniform distribution of drug and endogenous nucleotide concentrations across the biopsy, which is unlikely.[42] However, isolating HIV target cells from vaginal and cervical tissue biopsies has resulted in incomplete pharmacokinetic data sets due to small, inconsistent cell yields[39], and previous publications have demonstrated linear relationship between isolated mucosal cells and tissue homogenate concentrations.[29]

Although we used pharmacokinetic data collected only from women, parent and metabolite colorectal drug concentrations overlapped with previously published data in men,[7] suggesting mucosal tissue differences are driven primarily by the drugs’ distribution characteristics rather than inherent sex differences. Since the risk of HIV transmission per act of unprotected receptive anal intercourse does not differ between MSM and heterosexuals[43], it is unlikely that biological differences alter HIV infectability in male vs female colorectal tissue. Thus, we believe it is reasonable to substantiate our colorectal model predictions with PrEP clinical trial results from MSM only populations. Lastly, our population pharmacokinetic model was generated using data from healthy volunteers in the absence of sexually transmitted diseases or conditions causing inflammation in the lower gastrointestinal tract and FGT. Active metabolite and endogenous nucleotide concentrations may be altered in a setting mucosal tissue
inflammation,[44] along with altered susceptibility to HIV infection and warrant further investigation.

In summary, this study is the first of its kind for HIV oral PrEP. By pairing phase I tissue concentration data with in vitro systems of HIV infection, we created a predictive population PK/PD model for PrEP which is consistent with clinical trial outcomes. Here we demonstrate proof of concept that a more complete characterization of the achievable and effective concentrations can be used to generate PK/PD models to optimize drug dosing prior to the initiation of phase III trials and quantify the effect of incomplete adherence on protective efficacy. We believe this approach can be applied to other drugs and combinations to inform clinical trial design and optimize resources within the HIV prevention field.
Footnote Page

Conflict of Interests/Disclosures: Angela Kashuba and her laboratory are part of the study teams for CAPRISA 004 and 008, FACTS 001, MTN 006, HPTN 066, FEM-PrEP, and CONRAD 113, 114, and 117. Grant funding from Gilead Sciences Inc. has been received by UNC and Angela Kashuba. No other authors have commercial interests that might pose a conflict of interest to disclose.

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Figure Legends

Figure 1 Dose Proportionality in Blood Plasma, PBMCs, and Mucosal Tissues

Figure 1. Mean (±Standard Error) AUC$_{0-48}$h for tenofovir (A), tenofovir diphosphate (TFVdp; B), emtricitabine (C), and emtricitabine triphosphate (FTCtp; D) in the female genital tract (FGT) tissue (Δ); colorectal tissue (○); or plasma (□) for tenofovir and emtricitabine, and (□) peripheral blood mononuclear cells (PBMCs) for TFVdp and FTCtp.

Figure 2 Tissue Endogenous Nucleotide Concentrations

Figure 2. Tissue endogenous nucleotide concentrations are plotted as median (solid line within each box), 25th to 75th percentile (box edges), and 10th to 90th percentile (error bar whiskers) for 47 women. (*) denotes p<0.05. Median deoxyadenosine triphosphate (dATP) concentrations (A) are 85% lower in colorectal tissue compared to cervical and vaginal tissue (p<0.05; N=47). Median deoxycytidine triphosphate (dCTP) concentrations (B) in colorectal tissue are 90% lower than cervical tissue and 80% lower than vaginal tissue (p<0.001; N=47).

Figure 3= In Vitro Concentration vs Response for TZM-bl and CD4$^+$ Cells

Figure 3. TZM-bl and stimulated, primary CD4$^+$ cells were incubated for 24hr in 0.03-35µM concentrations of tenofovir and emtricitabine. Intracellular active metabolite and endogenous nucleotide (EN) concentrations were quantified at the time of challenge with HIV-1JR-CSF (TZM-bl cells) or psuedovirus with HIV-1 JR-CSF envelope (CD4$^+$ cells). For TZM-bl cells N=34 TFVdp:dATP and 41 FTCtp:dCTP samples collected across at least 2 independent experiments (6 of 72 samples were below the limit of quantification; BLQ). For CD4$^+$ cells N=14 TFVdp:dATP and 27 FTCtp:dCTP samples collected across at least 2 independent experiments (2 of 42 samples were BLQ). Solid lines represent the median regression line of the Emax model.
The dashed reference line indicates 90% inhibition of HIV infection and the dotted represents 50% inhibition. The fifty percent effective concentration (EC$_{50}$) ratio for tenofovir diphosphate (TFVdp):dATP (A) was 0.010 (SE=0.001, p<0.001) with a Hill slope (H) of 1.02 (SE=0.09, p<0.001) in TZM-bl cells (○) and 0.086 (SE=0.011, p<0.001) with an H of 1.81 (SE=0.39, p<0.001) in CD4+ cells (▲). The EC$_{50}$ ratio for emtricitabine triphosphate (FTCtp):dCTP (B) was 0.059 (SE 0.004, p<0.001) with an H of 1.42 (SE=0.11, p<0.001) in TZM-bl cells (○) and 0.022 (SE=0.005, p<0.001) with an H of 1.88 (SE=0.67, p<0.05) in CD4+ cells (▲).

**Figure 4. Time to Protection and Minimally Effective PrEP Dosing**

Figure 4 reports pharmacokinetic/pharmacodynamic (PK/PD) simulations for each tissue type: colorectal (A,B) and lower female genital tract (FGT; C,D). The percent of the simulated population achieving the EC$_{90}$ TFVdp:dATP, FTCtp:dCTP, or combination ratios derived from the in vitro PK/PD relationship in CD4$^+$ cells is plotted over the dosing interval for the first 10 daily doses (A,C) of tenofovir disoproxil fumarate (TDF; dashed line), emtricitabine (FTC; dotted line), or the fixed dose combination (TDF+FTC; solid line). The percent of the population achieving these EC$_{90}$ ratios at the end of the dosing interval under steady state conditions with 1 to 7 doses/week of TDF, FTC, or TDF+FTC are stratified by tissue (B,D). We predict that the maximal percent of the population achieved EC$_{90}$ ratios by the third daily dose of TDF+FTC in colorectal and FGT tissue after beginning PrEP. Consistently using 7 doses/week of TDF+FTC will achieve EC$_{90}$ ratios in 100% of the population in the FGT and colorectal tissue. Only 65% of the population using 2 doses/week of TDF+FTC achieve target exposure in the FGT tissue, whereas $\geq$95% using 2 doses/week of either TDF or TDF+FTC achieve target exposure in colorectal tissue.
Figure 5 Protection with Pericoital PrEP Dosing

Figure 5 reports pharmacokinetic/pharmacodynamic (PK/PD) simulations for the Ipergay dosing regimen (#2 tablets 2 to 24hrs before coitus (solid vertical line), #1 tablet 24hrs after coitus, #1 tablet 48hrs after coitus) for tenofovir disoproxil fumarate (TDF; solid line) and the fixed dose combination of tenofovir disoproxil fumarate+emtricitabine (TDF+FTC; dashed line). The percent of the simulated population achieving the EC$_{90}$ TFVdp:dATP ± FTCtp:dCTP derived from the in vitro PK/PD relationship in CD4$^+$ cells over 14 days following a single act of coitus in colorectal (A,B) and lower female genital tract (FGT) tissue (C,D). PK/PD simulations are reported assuming the first dose was administered 24hrs (A,C) or 2hrs (B,D) before coitus. We predict the maximal percent of the population achieving EC$_{90}$ ratios in colorectal tissue over a 240hr postcoital window is achieved by initiating the Ipergay dosing 24hrs before coitus. Dosing at 24hrs or 2hrs before coitus did not appear to alter the percent of the population achieving target exposure in the FGT tissue over a 72hr postcoital window.
Table 1. Demographics and Adverse Events

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Tenofovir disoproxil fumarate (N=25)</th>
<th>Emtricitabine (N=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27 (22.75-31.25)</td>
<td>22 (21-27)</td>
</tr>
<tr>
<td>Weight (kg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.4 (60.4-76.8)</td>
<td>62.8 (57.7-72.3)</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.1 (21.6-26.9)</td>
<td>22.5 (20.8-26.5)</td>
</tr>
<tr>
<td>Race&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>16 (64)</td>
<td>18 (75)</td>
</tr>
<tr>
<td>African American</td>
<td>8 (32)</td>
<td>4 (16.7)</td>
</tr>
<tr>
<td>Asian American</td>
<td>1 (4)</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td>American Indian</td>
<td>0 (0)</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td>Adverse Event&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>1 (4)</td>
<td>5 (20.8)</td>
</tr>
<tr>
<td>Nausea</td>
<td>2 (8)</td>
<td>0</td>
</tr>
<tr>
<td>Early menses</td>
<td>1 (4)</td>
<td>0</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1 (4)</td>
<td>0</td>
</tr>
<tr>
<td>Bowel disturbances</td>
<td>1 (4)</td>
<td>1 (4.2)</td>
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<td>Elevated transaminases</td>
<td>0</td>
<td>1 (4.2)</td>
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<tr>
<td>Pelvic cramps</td>
<td>0</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td>Post nasal drip</td>
<td>0</td>
<td>1 (4.2)</td>
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<tr>
<td>Vaginal dryness</td>
<td>0</td>
<td>1 (4.2)</td>
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<tr>
<td>Ear infection</td>
<td>1 (4)</td>
<td>0</td>
</tr>
<tr>
<td>Viral pharyngitis</td>
<td>1 (4)</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data summarized as median (Interquartile range)

<sup>b</sup> Data summarized as number (percentage)
Table 2. Dose Proportionality Statistics

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Analyte</th>
<th>Slope ($\hat{\beta}_1$)$^a$</th>
<th>90% Confidence Interval</th>
<th>$r^2$ (p value)</th>
<th>Dose Proportional$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>Emtricitabine</td>
<td>0.84</td>
<td>0.70, 0.97</td>
<td>0.85 (&lt;0.0001)</td>
<td>Yes</td>
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<tr>
<td>Plasma</td>
<td>Tenofovir</td>
<td>0.77</td>
<td>0.63, 0.92</td>
<td>0.80 (&lt;0.0001)</td>
<td>No</td>
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<tr>
<td>PBMC</td>
<td>FTCtp</td>
<td>0.19</td>
<td>0.012, 0.37</td>
<td>0.13 (0.09)</td>
<td>No</td>
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<tr>
<td>PBMC</td>
<td>TFVdp</td>
<td>1.07</td>
<td>0.91, 1.24</td>
<td>0.83 (&lt;0.0001)</td>
<td>Yes</td>
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<tr>
<td>FGT</td>
<td>Emtricitabine</td>
<td>0.91</td>
<td>0.78, 1.05</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>FGT</td>
<td>FTCtp</td>
<td>0.12</td>
<td>-0.29, 0.53</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>FGT</td>
<td>Tenofovir</td>
<td>0.77</td>
<td>0.46, 1.09</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>FGT</td>
<td>TFVdp</td>
<td>0.93</td>
<td>0.14, 1.72</td>
<td>Not applicable; Sparsely</td>
<td>No</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>sampled data</td>
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<tr>
<td>Colorectal</td>
<td>Emtricitabine</td>
<td>1.24</td>
<td>-0.29, 2.77</td>
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<td>No</td>
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<tr>
<td>Colorectal</td>
<td>FTCtp</td>
<td>0.22</td>
<td>-0.23, 0.67</td>
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<td>No</td>
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<td>Colorectal</td>
<td>Tenofovir</td>
<td>2.15</td>
<td>0.31, 4.00</td>
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<td>No</td>
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<tr>
<td>Colorectal</td>
<td>TFVdp</td>
<td>0.35</td>
<td>-1.10, 1.80</td>
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<td>No</td>
</tr>
</tbody>
</table>

$^a \hat{\beta}_1=$ regression line slope for log transformed AUC$_{0-48}$ vs log transformed dose.

$^b$ Perfect dose proportionality $\hat{\beta}_1 = 1$ therefore dose proportionality declared if the 90% confidence interval within 0.64, 1.36

FGT=female genital tract; FTCtp=emtricitabine triphosphate; PBMC=peripheral blood mononuclear cells; TFVdp=tenofovir diphosphate