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Methotrexate decreases tenofovir exposure in antiretroviral-suppressed individuals living with HIV

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Abstract

Background: To mitigate increased risk of premature cardiovascular disease in antiretroviral therapy (ART) suppressed adults living with HIV (PWH), low dose methotrexate (LDMTX) was evaluated in a multicenter randomized placebo controlled clinical trial of 176 PWH taking various ART regimens (ACTG A5314). Given shared methotrexate (MTX) and tenofovir (TFV) pharmacokinetic (PK) pathways, a substudy was carried out to investigate whether LDMTX alters TFV exposure.

Methods: Adults virally suppressed on ART for >24 weeks were randomized to LDMTX or placebo. The first 66 participants taking a tenofovir disoproxil fumarate-containing regimen underwent intensive PK sampling over 24 h following the second dose of LDMTX 10 mg or placebo. TFV and MTX levels were quantified using validated mass spectrometry methods. TFV PK between LDMTX and placebo groups were compared and MTX PK was characterized.

Results: Forty-eight participants completed this substudy (n=20 on LDMTX and 28 on placebo). Baseline characteristics were balanced except for PI-use (25% in LDMTX and 43% in placebo groups). For TFV, AUC_6 (primary endpoint), and $AUC_{24,imputed}$, C_{max}, and C_{min} (secondary endpoints) were on average 22%, and 24%, 27%, and 31% less in the LDMTX versus placebo groups, with reductions in secondary endpoints reaching statistical significance. Additional analyses suggested a greater reduction in the absence of PI although not significant.

Conclusion: Lower TFV $AUC_{24,imputed}$ and C_{max} indicates that LDMTX reduces TFV exposure in PWH. However, this change was modest, not warranting a change in TFV dosing at this time. Further studies of TFV PK with LDMTX, especially without PI co-administration, are warranted.

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Keywords

tenofovir; methotrexate; pharmacokinetics; drug interactions; transporters

Introduction

A recent study of the AIDS Clinical Trials Group (ACTG), A5314, investigated the use of low dose methotrexate (LDMTX) to reduce inflammation and also improve endothelial function associated with chronic HIV infection[1]. This study, a phase II trial, investigated the safety and efficacy of LDMTX in participants with adequately controlled HIV on antiretroviral therapy (ART). In this pharmacokinetic (PK) substudy, we hypothesized that tenofovir (TFV), given as tenofovir disoproxil fumarate (TDF), a common component of ART regimens, may be subject to drug-drug interactions with methotrexate (MTX) given that both drugs are renal organic ion transporter (OAT) 1 and OAT3 substrates.

MTX is primarily eliminated renally unchanged via filtration and active secretion via OAT1 and OAT3[2,3] but exhibits other complex pharmacological characteristics, including variable absorption (t_{max} 0.7–4 h) and saturable dose-dependent absorption (28 to 88% bioavailability)[5,6]. It is also significantly metabolized by intestinal flora and intracellularly to more active polyglutamate derivatives retained in the cells until reverse conversion for elimination[5]. Coadministration of OAT-transported substrates, such as penicillins[7,8] and ciprofloxacin[9] have resulted in increased, potentially toxic, MTX levels. MTX has not been identified a perpetrator of renal transport-related drug-drug interactions[10], but studies are limited.

Similar to MTX, TFV is also eliminated by renal filtration and active secretion by OAT1 and OAT3[11,12]. TFV, administered as TDF, is poorly absorbed (approximately 25% bioavailability)[13]. For some known TFV drug interactions, definitive mechanisms are unclear. In the case of higher TFV with protease inhibitors (e.g. atazanavir[14]), inhibition at the apical membrane resulting in higher TFV accumulation in proximal tubule cells has been proposed. For TFV increasing raltegravir exposure[15], OAT1 inhibition has been suggested as a potential mechanism[16].

For MTX, little is known regarding the use of low dose oral MTX in the context of ART. One study observed no difference in MTX half-life following high dose intravenous administration to ART treated PWH[17], but did not investigate the impact of MTX on TFV PK.

Such interactions may result in increased TFV or MTX levels, which may result in TFV-mediated renal toxicity via accumulation in renal proximal tubule cells or toxic MTX exposure. Unexpected interactions that may decrease exposure of TFV or MTX, could potentiate loss of viral suppression or loss of MTX anti-inflammatory efficacy, respectively.

We therefore sought to evaluate the potential drug-drug interaction between LDMTX and TFV in people living with HIV (PWH). In this A5314 substudy enrolling TFV-treated participants, intensive PK samples were collected and analyzed for TFV and MTX

concentrations. Using PK and statistical analyses, we compared TFV exposure between those receiving active MTX to those receiving placebo and characterized low dose MTX exposure in the context of TFV-containing ART.

Methods

A5314 study design

A5314 is a phase II double blind, randomized placebo-controlled trial assessing the safety and efficacy of LDMTX on endothelial function and inflammation in PWH who have been virologically suppressed with continuous ART (NCT01949116)[1]. The study included men and women at least 40 years of age, with or at risk for atherosclerotic cardiovascular disease with ART-suppressed HIV (CD4+ T-cell count ≥ 400 cells/mm3 and HIV-1 RNA level<40 copies/mL for at least 24 weeks prior to study entry). Participants who met the enrollment criteria were randomized 1:1 to LDMTX or placebo. From entry through week 1 (lead-in period), participants took 5 mg once weekly by mouth of either MTX or placebo. Participants were then titrated to 10mg/week over 12 weeks, at which point they received the maximum dose of 15mg/week until week 24. If a participant did not meet the criteria for dose escalation at the protocol-defined dose escalation time, then the participant remained on his/her current dose until the next study visit at which time the participant was re-evaluated for dose escalation. All participants received 1 mg folic acid daily from study entry until 4 weeks after completion of study treatment, regardless of randomization.

PK Study Design

Participants were required to be on steady state TFV (defined as continuous ART for ≥24 weeks before enrollment without any changes to their basic regimen in the prior 12 weeks) and self-reported adherence for the last 4 doses. Intensive PK sampling was performed after the second dose of MTX (10 mg). LDMTX (or placebo) and TFV were administered at the same time and serial sampling was carried out at 0 (pre-dose), 0.5, 1, 2, 4, and 6 hours post-dosing (protocol version 2.0) for analysis of both TFV and MTX (for those on active drug) levels; under version 1.0 of the protocol some participants were also sampled at 8, 12 and 24 hours post-dosing. The reason for the 6 hour sampling was to enhance enrollment by limiting the time commitment for the study, but to still allow intensive PK sampling during the day while clinics remained open.

A sample size of approximately 21 evaluable participants per treatment group was chosen to provide 95% power to detect a clinically relevant 40% increase in TFV AUC (in LDMTX versus placebo arms) with a relaxed type-one error rate (1-sided 5%). Given the absence of appropriate control within the A5314, analysis plans for MTX exposure involved a simple characterization of the MTX AUC to be compared descriptively against historical controls.

MTX and TFV Assay Development and Sample Quantification

For study participants randomized to placebo, plasma samples were assayed only for TFV and not MTX while those randomized to LDMTX were analyzed for both TFV and MTX using validated liquid chromatography coupled with tandem mass spectrometry methods (LC-MS/MS). MTX was fortified with a deuterated internal standard, and extracted from 50

μL of plasma by protein precipitation with acetonitrile (ACN). The extracted samples were separated on an Agilent® Zorbax XDB-C_8 high performance liquid chromatography (HPLC) column (2.1×50mm, 5μm), and detected on a Sciex API5000 mass spectrometer. The method had a lower limit of quantitation (LLOQ) of 5 ng/mL, with a calibration range of 5–500 ng/mL. During sample analysis, the coefficient of variation (CV) for quality control samples (QC) ranged from 3.15% to 4.39%. Similar to MTX, TFV was fortified with a deuterated internal standard, and then 50 μL of sample was extracted by protein precipitation with ACN. The sample extracts were separated on a Phenomenex® Synergi Polar-RP HPLC column (150 × 2.0mm, 4μm), then detected on a Sciex API 5000 mass spectrometer. The LLOQ for TFV was 5 ng/mL, with a calibration range of 5–1000 ng/mL. The CV of the QC during TFV sample analysis ranged from 5.18% to 8.36%.

PK and Statistical Analysis

PK parameter outcomes included the area under the plasma concentration vs. time curve (AUC), and peak concentration (C_{max}) for MTX and TFV, and the trough concentration (C_{min}) for TFV. Parameters were estimated using non-compartmental analysis in WinNonlin v.6.2.1® (Certara L.P., Princeton, NJ, USA). For AUC calculations, samples below the LLOQ were treated as missing except for the pre-peak TFV or MTX concentrations, which was set to 0 if below the LLOQ. AUC was calculated using the linear up-log down trapezoidal rule from 0 to 6 h (AUC_6) and 0 to 24 h (AUC_{24} and AUC_{24i}, where AUC_{24} was available only in participants enrolled under Version 1.0 and AUC_{24i} was estimated for all participants using the pre-dose concentration as the imputed 24 h TFV concentration for participants enrolled under Version 2.0). Imputed concentrations assume there is no difference between the concentrations between 0 and 24 h for steady state dosing. For TFV, C_{min} was calculated from the raw data at time 0.

AUC_6 was the primary endpoint, while all other PK parameters (AUC_{24}, AUC_{24i}, C_{max}, C_{min}) were secondary endpoints for TFV exposure. The primary endpoint for MTX was AUC_6 and the secondary endpoint was C_{min}. TFV PK parameters were compared between placebo and LDMTX treatment groups, while MTX PK was characterized only in the context of TFV-containing ART. PK parameters were summarized using geometric means with 90% confidence intervals; imputed zero concentrations were set to 0.1 ng/mL to facilitate log transformation. Distributions were compared between LDMTX and placebo groups using two sample t-tests with unequal variance and were interpreted at the 10% nominal level of significance (two-sided test) without adjustment for multiple comparisons. This change to the analysis plan specified in the protocol (use of a 10% two-sided test versus a 5% one-sided test) was made prior to review of the data. All tests were performed on natural log-transformed PK parameters. Statistical analyses were conducted with SAS Version 9.4 (SAS Institute Inc, Cary, North Carolina, USA).

Results

Participant Demographics

Of the 66 participants enrolled in the substudy, 18 were excluded from PK analysis due to missed doses of TFV or failure to meet protocol criteria for dose-escalation to 10 mg of

MTX at the time of the substudy. Forty-eight participants completed PK sampling (n=20 on LDMTX, n=28 on placebo; a subset of participants were sampled through 24 hours: n=7 on LDMTX and n=10 on placebo); all were taking TFV in the form of TDF. Participants were 92% male, 48% white and 46% black; characteristics were balanced across treatment arms with the exception of concomitant protease inhibitor (PI)-use (25% LDMTX, 43% placebo). Complete demographic information for evaluable participants in PK substudy is presented in Table 1.

PK Results

Effect of MTX on TFV PK parameters—Table 2 details the descriptive statistics of TFV PK parameters by treatment group. The geometric mean (GM) (90% CI) for the primary endpoint, TFV AUC_6, was 967 (802, 1166) ng·h/mL for the LDMTX group and 1239 (1105, 1390) ng·h/mL for placebo (geometric mean ratio (GMR)= 0.78, 90% CI [0.64, 0.96], p=0.06). Similar results were observed for AUC_{24} (GMR=0.64, 90% CI [0.45, 0.91], p=0.08) and AUC_{24i} (GMR=0.76, 90% CI [0.61, 0.93], p=0.033). In addition, mean C_{max} was lower in the LDMTX group versus the placebo group (GM =231 ng/mL versus 315 ng/mL, GMR=0.73, 90% CI [0.60, 0.90], p= 0.027). Trough TFV concentrations (C_{min}) did not differ between arms (GMR=0.69, 90% CI [0.34, 1.40], p=0.39). Figure 1 depicts the average concentration time profile of TFV with and without MTX co-administration.

Due to the interaction between TFV and PIs and the observed treatment group imbalance, a sub-group analysis by concomitant PI use was performed. This analysissuggested lower TFV concentrations in the presence of LDMTX compared to placebo when taken in conjunction with a non PI-based regimen; this difference was not apparent in the context of co-administration with a PI regimen (Figure 2). Specifically, a greater effect of MTX on TFV exposure was observed for participants who were not on PIs (non PI) for LDMTX compared to placebo (C_{max} GMR_{nonPI}=0.7, 90% CI [0.53, 0.93], p=0.045; AUC_6 GMR_{nonPI} =0.76, 90% CI [0.58, 0.98], p=0.08; AUC_{24i} GMR_{nonPI}=0.75, 90% CI [0.58, 0.98], p=0.08). While a formal interaction test was not statistically significant (p>0.3), this test is underpowered given the small study sample.

MTX PK in the context of TFV- containing ART—MTX was characterized in the context of TFV. MTX AUC_6 was estimated to be 492 (434, 558) ng·h/mL, and C_{max} was 144 (127, 164) ng/mL. The geometric mean concentration-time profiles for MTX in the presence of TFV are shown in Supplemental Figure 1. MTX exposure did not appear to differ by PI use on visual evaluation.

Discussion

Common renal elimination transporter pathways of TFV and MTX raise concern for potential drug-drug interactions. In this study, we investigated the impact of LDMTX on TFV PK via intensive venous sampling in a subset of participants chronically suppressed with TFV-containing ART who were randomized to either active LDMTX or placebo as part of the A5314 study[1]. Our results demonstrate decreased TFV exposure in the presence of LDMTX, including a 22% reduction in the geometric mean AUC_6. Similar results were seen for AUC_{24}, AUC_{24i}, and C_{max} (with 36%, 24%, and 27% reductions, respectively). While

the differences in GMR for some PK parameters did not reach statistical significance, these results demonstrate an overall trend towards decreased TFV exposure in the presence of MTX. However this magnitude of decrease in TFV exposure is likely not clinically significant towards providing adequate viral suppression. As reported for the parent trial[1], very few participants in the trial as a whole experienced a HIV-1 RNA level above the assay limit of quantification (40 copies/mL), and all were evenly distributed by treatment group. Among participants with measures above the assay limit of quantification, none had confirmed viral load failures (HIV-1 RNA >200 copies/mL). Visually, the terminal slopes appear identical and rate of absorption similar. In support of this observation, reductions in TFV exposure are not a result of inhibition of renal transporters during excretion, and are likely attributable to decreased absorption, as supported by a significant decrease in TFV C_{max} with LDMTX. Within the placebo arm, TFV C_{max} averaged 315 ng/mL, consistent with published TFV exposure[18].

Within PI subgroups, comparisons between study arms showed that TFV AUC_6, AUC_{24i}, and C_{max} trended toward higher values in participants on PIs, consistent with a previous report[19,20]. This potential PI based-TFV interaction is likely driven by increased absorption by PI-related inhibition of P-glycoprotein (P-gp) and intestinal esterase[20–22], resulting in higher TFV exposure estimates in the context of PIs which partially compensates for the overall lower TFV exposure measured during MTX co-administration. This trend toward lower TFV exposure is likely driven by inclusion of five participants not receiving PIs in the LDMTX group who exhibited particularly low TFV exposure. These participants were demographically similar to the PK substudy population with consistent body mass indices, age, creatinine clearance and mixed with regards to race. Overall when the TFV PK parameters are stratified by use of concomitant PIs, a modest shift is seen in the distributions in the absence of PIs; however, this shift is not apparent in the co-administration of concomitant PIs and LDMTX.

MTX levels were also quantified using a precise analytical method but no statistical comparisons were possible due to lack of a control group not receiving TFV. However, comparisons were made to parameters published previously for low dose MTX (Table 3). One would anticipate lower peak concentrations for intramuscular (IM) versus oral (PO) dosing and higher peak concentrations for 10 mg versus 7.5 mg PO doses. The MTX C_{max} observed in this study was about 40–60 and 34% lower than published studies of weekly 10 mg IM dosing [23–25] and weekly 7.5 mg PO dosing [26], respectively. Of note, studies among individuals with rheumatoid arthritis report a wide range of bioavailability of oral methotrexate ranging from 20–100% [27,28]. It is also possible that that TFV and/or HIV disease may decrease MTX exposure. The earlier studies used florescence polarization immunoassays (FPIA) or radioimmunoassay; a recent comparison of FPIA to LC-MS/MS indicates FPIA overestimated concentrations in this range[29], which may explain the discrepancy with prior PK estimates. One study, using a homogenous enzymatic immunoassay, reported no effect of TFV on MTX elimination[17]. However, that study examined high doses of intravenous MTX, supporting that a potential interaction is likely driven by an absorption process, rather than elimination. Further studies with a non-TFV control are needed to confirm the effect of TFV on MTX exposure.

One of the limitations of study A5314 was that the targeted LDMTX was on the low end of the dose range that had been effective in reducing cardiovascular events in larger cohort studies of MTX[30–34]. While exposure-response analysis was not the focus of the primary study, it is possible that if the 57/86 participants assigned to LDMTX who were taking TDF had lower than anticipated exposure to MTX, its effects on the primary efficacy and safety endpoints may have been reduced, contributing to decreased power to detect differences between arms.

Understanding the underlying mechanisms of TFV- and MTX-mediated drug-drug interactions and toxicities, particularly the role of renal and intestinal transporters, continues to evolve and is a rich area of research and scientific debate. TFV is a substrate of renal transporters (OAT1, OAT3, multidrug resistance protein[MRP] 4) and TDF is subject to intestinal transport via P-gp, and likely MRP4. MRP2's role in TFV PK has been debated in the literature[21,35]. More recently, TFV was identified as a substrate of MRP8 for which MTX is also a known substrate[36]. Other transporters, such as MRP7, have been associated with renal injury but mechanistic studies are lacking[37]. MTX is a substrate for numerous transporters[38]; those shared with known or potential TFV or TDF pathways include OAT1 and OAT3[2,3], MRP2[39], MRP4[39], MRP8[36], and P-gp[40].

Of these pathways MRP4, mechanistically, has the most potential for a TDF-MTX PK interaction. MRP4 is an efflux transporter; although since it's located on the basolateral side of enterocytes, it ultimately facilitates drug entry to the blood and serves to mediate intestinal basolateral influx of TDF and MTX. The observed results of this study would be supported by this mechanism given lower C_{max} for TFV in the presence of MTX and for MTX as compared to prior studies.

While the role of intestinal MRP4 in TDF absorption has not been well described, it was found to be a major contributor to adefovir dipivoxil uptake[41], a compound similar structurally and pharmacokinetically to TDF. Furthermore, Vitamin D_3, a known inducer of MRP4 expression, enhanced adefovir exposure in rats[42]. Mouse models have also demonstrated the importance of MRP4 in cefadroxil absorption[43] and dasatinib absorption and efficacy[44]. *In vitro* and *in situ* interaction studies of TDF on atazanavir absorption supported clinical findings that TDF decreases atazanavir bioavailability; the authors hypothesized that OATP or MRP-mediated absorption pathways were likely involved[45].

While MRP4 present in proximal renal cells is considered important in TFV-mediated kidney injury[46], intestinal MRP4 may be more so subject to saturation by MTX and TDF, given its lower expression frequency[47]. Although preclinical experiments in MRP4 knockout mice failed to show importance of MRP4 in MTX absorption[48], *in vitro* interaction assays demonstrated MRP4-mediated interactions between MTX and nonsteroidal anti-inflammatory drugs[39]. There are known inconsistencies in drug transporter expression in *in vitro* and preclinical models versus in human tissues[49] limiting clinical translation of preclinical findings. MRP4 is expressed in the human jejunum[49], the likely site of MTX absorption[5,50].

Further, genetic polymorphisms in transporter expression may have contributed to observed between-subject PK variability. Both MTX and TFV have documented polymorphisms that contribute to differences in PK, efficacy, or toxicity in varying patient populations and indications[51]. For instance, genotype differences in MRP4 have been linked to altered TFV clearance in PWH and differential MTX plasma exposure in pediatric patients being treated for acute lymphoblastic leukemia[19,52].

Aside from transporters, an emerging field, the gastrointestinal microbiome, which may also play a key role in HIV disease pathogenesis[53], has been shown to metabolize and/or be altered by exposure to both TDF[54] and MTX [55,56]. Future studies may investigate the interplay between the microbiome and pharmacokinetics of multiple coadministered drugs.

The clinical implications of these results on the use of tenofovir alafenamide (TAF) are unclear. Unlike TDF, TAF is relatively stable in plasma and exhibits lower TFV plasma levels and higher intracellular tenofovir diphosphate levels than TDF. While sharing some transport pathways with TDF (i.e. P-gp and breast cancer resistance protein [BCRP]), TAF is additionally a substrate of OATP1B1 and OATP1B3, but not subject to OAT1 or OAT3 transport[57,58]. Current literature does not indicate whether TAF is a substrate of MRP4. Given these differences, separate preclinical and/or clinical PK investigations of TAF and its potential in transporter-mediated interactions with MTX are warranted.

In summary, LDMTX resulted in modest reductions in TFV exposure that are not expected to be clinically significant for maintenance of HIV-1 viral suppression. However, these decreases appear driven by a subset of participants who were not on PIs, which indicates a change in TFV dosing is not warranted for those taking PIs. Further confirmatory and mechanistic studies of TFV PK in the setting of LDMTX without PI co-administration are warranted. Although MTX exposure was only characterized in the context of TFV co-administration, exposure was lower than anticipated from prior reports, which may have impacted effects of LDMTX on cardiovascular outcomes. This study will help inform dosing during MTX-TFV co-administration particularly for PWH who have rheumatoid arthritis or psoriasis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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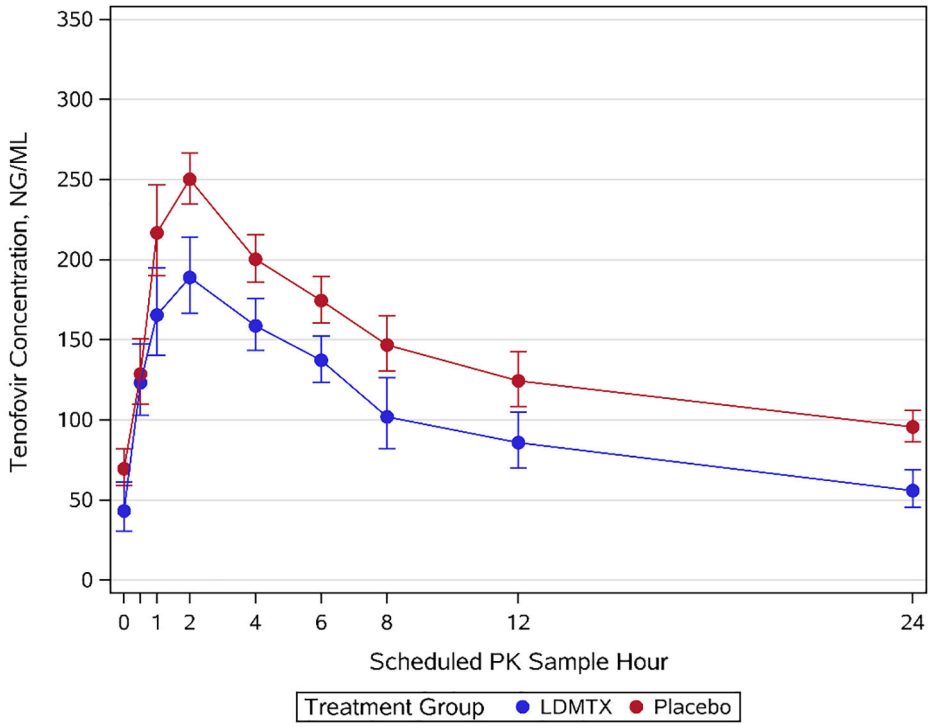

Figure 1.

Geometric mean plasma concentration-time profile of TFV. Blue line, TFV with LDMTX; red line, TFV with placebo. Error bars indicate standard error.

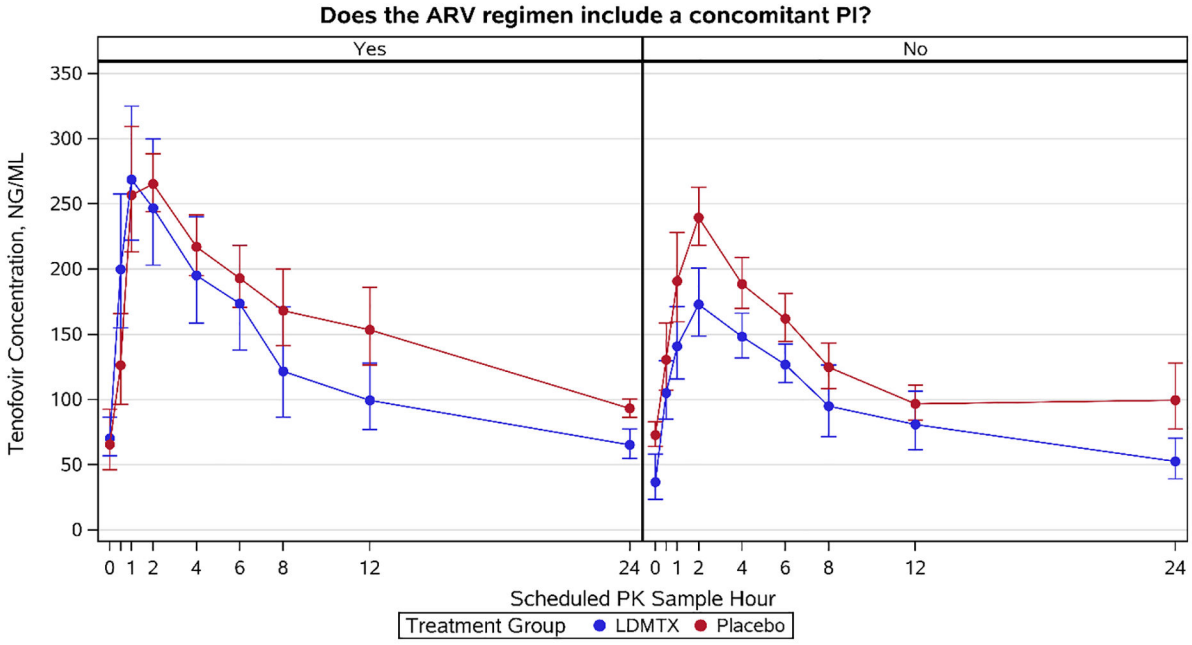

Figure 2.

Geometric mean plasma concentration-time profile of TFV in the context of LDMTX. Blue line, TFV with LDMTX; red line, TFV with placebo. Error bars indicate standard error.

Table 1.

Demographic Information of the participants in the PK substudy. SD: standard deviation

			Treatment Group	
Characteristic		**Total (N=48)**	**LDMTX (N=20)**	**Placebo (N=28)**
Sex	M	44 (92%)	17 (85%)	27 (96%)
	F	4 (8%)	3 (15%)	1 (4%)
Race	Black or African American	22 (46%)	9 (45%)	13 (46%)
	White	24 (50%)	10 (50%)	14 (50%)
	More than One Race	1 (2%)	0 (0%)	1 (4%)
	Unknown	1 (2%)	1 (5%)	0 (0%)
Race/Ethnicity	White Non-Hispanic	23 (48%)	9 (45%)	14 (50%)
	Black Non-Hispanic	22 (46%)	9 (45%)	13 (46%)
	Hispanic (Regardless of Race)	2 (4%)	2 (10%)	0 (0%)
	More than one race	1 (2%)	0 (0%)	1 (4%)
Age (years)	Median (Q1-Q3)	54 (49–59)	56 (52–61)	52 (49–57)
BMI (kg/m^2)	# missing	2	0	2
	Median (Q1-Q3)	27.5 (24.3–31.1)	29.1 (24.5–31.2)	27.4 (24.1–29.8)
Calculated Creatinine Clearance (mL/min)	Median (Q1-Q3)	98 (84–115)	110 (86–116)	96 (81–110)
Entry CD4+ Cell Count ($cells/mm^3$)	Median (Q1-Q3)	731 (625–946)	687 (539–968)	756 (649–918)
Does the ARV regimen include a concomitant NNRTI	Yes	24 (50%)	12 (60%)	12 (43%)
Does the ARV regimen include a concomitant PI	Yes	17 (35%)	5 (25%)	12 (43%)
Does the ARV regimen include a concomitant II	Yes	10 (21%)	3 (15%)	7 (25%)

Table 2.

PK parameters for TFV after co-administration with LDMTX. Data represent Geometric mean (GM) with 90% CIs, except for geometric mean ratio (GMR) of LDMTX/placebo.

	LDMTX (n=20)	Placebo (n=28)	LDMTX/Placebo	
	GM (90% CI)	GM (90% CI)	GMR (90% CI)	p-value
AUC_6 (ng•h/mL)	967 (802, 1166)	1239 (1105, 1390)	0.78 (0.64, 0.96)	0.06
PI-based ART	1275 (874, 1859)	1343 (1127, 1600)	0.95 (0.68, 1.32)	0.80
Non-PI-based ART	882 (706, 1101)	1167 (992, 1372)	0.76 (0.58, 0.98)	0.08
AUC_{24} (ng•h/mL) *	2235 (1511, 3306)	3481 (2948, 4110)	0.64 (0.45, 0.91)	0.08
AUC_{24i} (ng•h/mL)	2647 (2218, 3160)	3503 (3087, 3975)	0.76 (0.61, 0.93)	0.033
PI-based ART	3331 (2202,5039)	3873 (3205, 4679)	0.86 (0.60, 1.23)	0.52
Non-PI-based ART	2453 (1995, 3014)	3249 (2718, 3883)	0.75 (0.58, 0.98)	0.08
C_{max} (ng/mL)	231 (188, 283)	315 (284, 349)	0.73 (0.60, 0.90)	0.027
PI-based ART	314 (234,420)	341 (291, 400)	0.92 (0.69, 1.22)	0.62
Non-PI-based ART	209 (162, 268)	296 (256, 343)	0.70 (0.53, 0.93)	0.045
C_{min} (ng/mL)	43 (24, 79)	63 (41, 97)	0.69 (0.34, 1.40)	0.39
PI-based ART	70 (45, 110)	51 (18, 146)	1.37 (0.27, 6.93)	0.62
Non-PI-based ART	37 (16, 82)	73 (58, 92)	0.50 (0.23, 1.11)	0.17

* n=7 for LDMTX and 10 for placebo. AUC, area under concentration-time curve, AUC_{24i}, AUC from 0 to 24 h with imputed 24 h concentration from 0 h, PI, protease inhibitor.

Table 3.

Comparison of methotrexate exposure in this study and published literature. Data represent Geometric mean (90% CIs)

Dose (mg)	Administration Route	C_{max} (ng/mL)	Reference
10	PO	144 (127, 164)	This study
7.5	PO	217 (180, 254)	Sinnett et al[26]
10	IM	233 (207, 259)	Combe et al[23]
10	IM	268 (237, 299)	Anaya et al[24]
10	IM	350 (253, 446)	Edelman et al[25]