Antenatal Intracellular Concentrations of Tenofovir Diphosphate and Emtricitabine Triphosphate and Associations Between Tenofovir Diphosphate and Severe Adverse Pregnancy Outcomes: IMPAACT-PROMISE (1077BF) Trial

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Background: In the Promoting Maternal and Infant Survival Everywhere (PROMISE) trial, tenofovir disoproxil fumarate (TDF) use was associated with moderate or severe adverse pregnancy/neonatal outcomes. This study characterized tenofovir diphosphate (TFV-DP) and emtricitabine triphosphate (FTC-TP) concentrations in dried blood spots (DBS) and assessed association between severe adverse pregnancy/neonatal outcomes and TFV-DP concentration.

Methods: Retrospective case–control study of PROMISE trial arm-C women randomized to receive TDF, FTC, and ritonavir-boosted lopinavir (LPV/r), who took at least 1 dose of TDF + FTC and had week-4 postrandomization DBS drawn before delivery. Cases, defined as severe adverse pregnancy/neonatal outcomes (very preterm delivery before 34 weeks of gestation, stillbirth ≥20 weeks of gestation, or infant death before 14 days-of-age), were matched to controls (1:2 ratio) by site and gestational age at entry. Week 4 and week 8 DBS samples were assayed for TFV-DP and FTC-TP by liquid chromatography and tandem mass spectrometry. Associations were tested using Wilcoxon rank test and conditional logistic regression.

Results: Of 447 PROMISE arm-C women, 33 met case definitions, and overall, 22 cases and 44 controls were analyzed. Median (interquartile range) concentrations of TFV-DP at weeks 4 and 8 were 706 (375–1023) fmol/punch and 806 (414–1265) fmol/punch, respectively. Odds ratio (95% confidence interval) for severe adverse pregnancy/neonatal outcome with natural log of TFV-DP concentrations as the predictor were 1.27 (0.74 to 2.18) and 1.74 (0.66 to 4.60) at weeks 4 and 8, respectively. Median (interquartile range) concentrations of FTC-TP at weeks 4 and 8 were 0.27 (0.05–0.36) pmol/punch and 0.29 (0.05–0.40) pmol/punch, respectively.

Conclusions: TFV-DP concentrations in DBS appeared not to be associated with severe adverse pregnancy/neonatal outcomes, although sample size was limited.

Key Words: tenofovir diphosphate (TDF), emtricitabine triphosphate (FTC-TP), DBS, severe adverse pregnancy/neonatal outcomes, HIV-infected African and Indian women

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INTRODUCTION

Tenofovir disoproxil fumarate (TDF), a potent nucleotide analogue reverse transcriptase inhibitor, is the mainstay of first-line treatment regimens for HIV and/or hepatitis B virus (HBV)–infected adults, including pregnant women, according to the World Health Organization (WHO) guidance.[1,2] Tenofovir-based treatment regimens are used to treat millions of people living with HIV and/or HBV throughout sub-Saharan Africa and around the world.[3,4]

Safety concerns of TDF use during pregnancy persist and efforts to address these research gaps have potential public health benefits. In the multicountry Promoting Maternal and Infant Survival Everywhere (PROMISE) randomization trial among African and Indian HIV-infected pregnant women, exposure to either TDF-based or zidovudine (ZDV)-based antiretroviral (ARV) therapy (ART) was significantly related to moderate adverse pregnancy outcomes, including preterm delivery (PTD) before 37 weeks of gestation and low birth weight below 2500 g when compared with antenatal ZDV alone exposure. When looking only at severe adverse pregnancy and neonatal outcomes, TDF-based ART was significantly associated with very PTD (vPTD) before 34 weeks of gestation, still birth (SB), and early infant death (EID) within 14 days of life but not very low birth weight below 1500 g when compared with ZDV-based ART.[5] Specifically, women randomized to receive TDF-based ART [TDF + emtricitabine (FTC) + ritonavir-boosted lopinavir (LPV/r)] compared with those in the zidovudine (ZDV)-based ART [ZDV + lamivudine (3TC) + LPV/r] arm had more than double the risk of vPTD before 34 weeks gestation (6.0% vs. 2.6%, $P = 0.04$) and were more than 4 times as likely to have EID (4.4% vs. 0.6%; $P = 0.001$). These findings were unexpected and stood in contrast to prior studies that reported reassuring outcomes with TDF use during pregnancy for the treatment of women with HIV and/or HBV and for HIV prevention (preexposure prophylaxis or PrEP).[6] A recent analysis of pregnancy outcomes by PROMISE ARV drug regimens in a combined data set from 2 United States perinatal HIV prevention cohorts demonstrated that adverse pregnancy outcome risks were not significantly different between TDF-based and ZDV-based ART groups.[7]

Both TDF- and ZDV-based PROMISE trial antepartum randomization ART regimens contained ritonavir-boosted lopinavir (LPV/r), which may increase TFV exposures.[8–11] Therefore, it is biologically plausible that a drug–drug interaction may have resulted in elevated TFV exposures for PROMISE women randomized to TDF-based ART. Higher TFV exposures have been associated with renal and bone toxicity in adults, and TDF use during pregnancy was also previously linked with decreases in bone mineral density in infants.[12] Thus, we hypothesized a priori that severe adverse pregnancy and neonatal outcomes in the PROMISE study, including vPTD, SB, and EID, might be associated with elevated antepartum TFV exposures. Pregnant women enrolled in the PROMISE study had dried blood spot (DBS) collections at select time points throughout the study, which permitted the unique ability to examine intracellular tenofovir diphosphate (TFV-DP) concentrations in these sample types. TFV-DP in DBS is a cumulative measure of TDF adherence over the previous several weeks to months of therapy owing to its long half-life in red blood cells (~17 days).[13,14] DBS assessments have been used to assess TDF/FTC adherence in multiple HIV prevention[15–18] and treatment studies,[19,20] and these measures have been examined in parallel with treatment efficacy, safety,[21] and drug–drug interaction[22,23] outcomes. However, there are currently no DBS data on TFV-DP levels in pregnant women living with HIV. Thus, this study sought to quantify intracellular TFV-DP and FTC-TP measured in stored DBS samples from selected PROMISE trial women and to determine relationships between TFV-DP concentrations in DBS and severe adverse pregnancy outcomes.

METHODS

Study Population

A retrospective case–control study included HIV-infected pregnant women sampled from the now completed PROMISE trial, which was supported by the National Institutes of Health supported International Maternal Pediatric Adolescents AIDS Clinical Trials (IMPAACT) Network. Eligible PROMISE study participants were enrolled across 14 sites in India, Malawi, South Africa, Tanzania, Uganda, Zambia, and Zimbabwe between 2011 and 2014. Written informed consent was obtained from all study participants. Institutional review boards based in the United States and the respective participating countries provided ethical research approval and oversight.

The IMPAACT-PROMISE 1077BF clinical trial (number NCT01061151: clinicaltrials.gov registry)[5,24] was an open-label, randomized, controlled trial that compared the relative efficacy and maternal/infant safety of 3 ARV drug regimens for the prevention of mother-to-child transmission of HIV. Three sequential antepartum and postpartum randomizations were conducted, and in the first randomization (Antepartum Component), enrolled participants were randomized to receive TDF + FTC + LPV/r, ZDV + 3TC + LPV/r, or ZDV only during pregnancy. Maternal follow-up assessments (clinical reviews and laboratory procedures including storage of blood samples for future use) were performed at trial entry; weeks 2, 4, 8, and 12 after entry and then every 4 weeks until delivery; at labor and delivery; and at the postpartum week 1 visit (held between 6 and 14 days postdelivery).

For this study, cases and controls were pregnant women from the Antepartum Component who were randomized to receive TDF + FTC + LPV/r from as early as 14 weeks of gestation through delivery, who received at least 1 dose of TDF + FTC while pregnant, and who had a week-4 DBS sample drawn before delivery. The available doses and formulations for TDF and FTC were 300- and 200-mg tablets, respectively. This study excluded women who had a spontaneous or induced abortion. The study used data collected through the administrative cutoff date of July 6, 2015. This was the day before participating research sites were instructed to inform all maternal participants about the START (Strategic Timing of Antiretroviral Treatment) trial results, which demonstrated statistically significant benefits of starting ART early compared with delayed initiation and offered ART to those who were not receiving it, thus ending the period of randomized trial data.[25]

Case Definitions

Severe adverse pregnancy/neonatal outcomes included: vPTD (birth before 34 weeks of gestation), SB at or after 20 weeks of gestation, or EID (before 14 days old). A woman was considered to be a "case" and to have met the composite

safety end point if any of the 3 adverse pregnancy outcomes (vPTD, SB, or EID) was observed. For multiple gestations, a woman was considered to have met the composite case definition if any of the adverse pregnancy/neonatal outcomes was observed on at least 1 fetus/infant. Gestational age at birth was based on one of the following determinations in hierarchical order: (1) pediatric evaluation using the Ballard newborn assessment done by trained pediatric staff,[26] (2) obstetrical evaluation, or (3) calculated gestational age at delivery based on gestational age at Antepartum Component entry and the date of delivery.

Case–Control Matching Criteria

Cases were matched to controls in a 1:2 ratio. To account for standard-of-care and timing and duration of in utero ARV drug exposures, each case had controls matched for each outcome by clinical research site and gestational age at study entry/randomization. A window of ±2 weeks was allowed. However, there were 2 vPTD cases for which a second control could not be identified within the ±2 weeks window, so the second controls were +6 weeks and +3 weeks for these cases. There were 2 cases in which the controls did not have 1:2 matching. One case (vPTD and EID outcomes) had only 1 control, whereas another case (vPTD and EID outcomes) had 3 controls (1 control applied to both vPTD and EID outcomes, 1 control applied only to the vPTD outcome, and 1 control applied only to EID outcome). The case with 3 controls was only for the composite end point; for the individual end point analyses, this case was matched 1:2.

Laboratory Procedures

Preparations of 50-μL DBS samples on Whatman 903 Cards were collected and stored at the respective site laboratories at −20°C or lower. DBS collections were randomly collected over the dosing interval, but given the long half-lives of TFV-DP and FTC-TP, similar levels would be expected over a 24-hour period. Selected samples were assayed using 3-mm punch sizes for TFV-DP and FTC-TP concentrations by liquid chromatography coupled with tandem mass spectrometry using previously described methods at the University of Colorado Antiviral Pharmacology Laboratory.[27] The lower limit of quantification (LLOQ) was 25 fmol/punch for TFV-DP and 0.1 pmol/punch for FTC-TP.

Statistical Methods

Separate analyses were done at weeks 4 and 8. Data were analyzed using SAS 9.4. The sample size for this substudy was determined by the number of TDF + FTC + LPV/r Antepartum Arm mothers who met criteria to be cases and had samples available for analysis. In cases where DBS drug concentrations were below LLOQ, these results were set to half the LLOQ (ie, 12.5 fmol/punch for TFV-DP and 0.05 pmol/punch for FTC-TP) for the primary analyses. As a sensitivity analysis for the comparison of DBS drug concentrations between cases and controls, drug concentration levels below LLOQ were set to 1 such that the ln (1) = 0. Summary statistics using median [interquartile range (IQR)] were calculated at weeks 4 and 8 to describe DBS drug concentration distributions; differences in concentrations between cases and controls were determined using the case concentration minus the average of its controls. Wilcoxon signed rank test (2 sided) was performed to assess differences in drug concentrations between cases and controls. Point and 95% confidence interval (CI) estimates for the odds ratio (OR) were calculated using the conditional logistic regression to assess the association between TFV-DP concentrations in DBS and the composite adverse pregnancy outcomes and each of its components. Given the skewed and widely spread distributions of the DBS drug concentrations, the natural log of the drug concentration was used in the conditional logistic analyses. The significance level was set at 0.05.

RESULTS

Study Profile

Out of 447 PROMISE mothers randomized to the TDF + FTC + LPV/r Antepartum Component Arm, 33 (7.38%) met criteria for being a "case"; of these, 23 received at least 4 weeks of TDF + FTC before delivery. Of these 23, 22 and 16 had drug concentration data at weeks 4 and 8, respectively. Ten cases (2 with vPTD and EID, 5 with PTD but no EID, and 3 with EID but without PTD) were excluded from the week-4 analyses for the following reasons: 1 did not receive any TDF + FTC, 6 delivered with less than 4 weeks of receipt of TDF + FTC, 3 had no week-4 DBS specimen; and 1 had week-4 DBS specimen but assay for drug concentrations was not done. An additional 7 cases were excluded from the week-8 analyses for the following reasons: 6 delivered with less than 8 weeks of receipt of TDF + FTC; and 1 had no week-8 DBS specimen. One case had only week-8 drug concentration data and was excluded from week-4 analyses but was included in week-8 analyses. Listings of the treatment and labor/delivery data for the 10 cases excluded from the week-4 analyses do not suggest any toxicity from TDF + FTC or any adverse event that would have resulted in early delivery. Overall, 66 women (22 cases and 44 matched controls) were included in the analyses (Fig. 1).

Tenofovir Diphosphate (TFV-DP) DBS Concentrations

One case and 2 controls at week 4, and none of the cases and one control at week 8, had a TFV-DP concentration below the LLOQ. For the composite safety end point, the overall median (IQR) TFV-DP concentration across cases and controls was 706 (375–1023) fmol/punch at week 4 and 806 (414–1265) fmol/punch at week 8 (Table 1). TFV-DP concentrations were comparable among cases compared with controls at the respective time points. The median (IQR) difference in TFV-DP concentrations among cases compared with controls was 15.5 (−232.0 to 142.5) fmol/punch ($P = 0.86$) at week 4 and 47.9 (−152.8 to 725.5) fmol/punch ($P = 0.38$) at week 8, respectively (Table 1 and Fig. 2). Similarly, analyses on SB and PTD at both time points and analysis on EID at week 8 showed no significant difference between the median TFV-DP concentration for the cases and controls ($P > 0.38$).

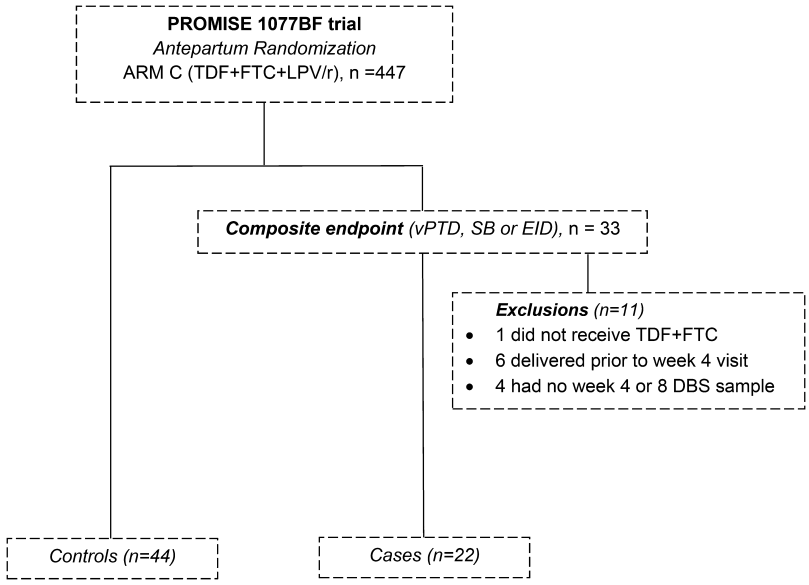

FIGURE 1. Flow diagram illustrating participant inclusion and exclusion. TDF, tenofovir; FTC, emtricitabine; vPTD, very preterm delivery (birth before 34 weeks of gestation); SB, stillbirth (at or after 20 weeks of gestation); or EID, early infant death (before 14 days old); DBS, dried blood spot; antepartum randomization (first 14 weeks of gestation or later) had 3 arms A, B, and C[5]; *"antepartum-Arm C"* had a combination of TDF, FTC, and LPV/r. Antepartum ARV drug regimens were taken through the postpartum randomization (6–14 days after delivery/birth). A woman randomized to *antepartum-Arm C* was considered a "case" and to have met the composite safety end point if any of the 3 adverse pregnancy outcomes (vPTD, SB, or EID) was observed. Cases were matched with 2 controls based on clinical research site and gestational age at entry to PROMISE antepartum component.

The OR (95% CI) of a woman having a severe adverse pregnancy/neonatal outcome (composite) per one natural log increase in concentration of TFV-DP was 1.27 (0.74 to 2.18) at week 4 and 1.74 (0.66 to 4.60) at week 8. Similarly, there was no significant association between TFV-DP concentrations and SB, PTD, or EID (Table 2). Study findings did not change when the values below LLOQ were set to ln(1) = 0.

Emtricitabine triphosphate (FTC-TP) DBS Concentrations

There were 8 cases (36%) and 11 controls (25%) at week 4 and 5 cases (31%) and 8 controls (25%) at week 8 who had FTC-TP concentrations below the LLOQ and values were set to 0.05 fmol/punch. For the composite severe safety end point, the overall median (IQR) FTC-TP concentration was 0.27 (0.05–0.36) pmol/punch at week 4 and 0.29 (0.05–0.40) pmol/punch at week 8 (Table 1). The median and IQR differences in FTC-TP concentrations between cases and controls at weeks 4 and 8 were 0.01 (IQR, −0.08 to 0.15) fmol/punch (P = 0.93) and 0.00 (IQR, −0.08 to 0.13) fmol/punch (P = 0.88), respectively. Most of the SB cases had a maternal FTC-TP concentration that was higher than the average concentration of their controls at weeks 4 and 8 (Fig. 3), although these differences were not significant (Table 1). Similarly, analyses on vPTD or EID found no significant

TABLE 1. DBS Drug Concentrations and Matched Case–Control Differences of TFV-DP and FTC-TP at Weeks 4 and 8 Post ART Initiation

	Week 4				Week 8			
Outcome	n	Concentration,* Median (IQR)	Difference,† Median (IQR)	P‡	n	Concentration, Median (IQR)	Difference,† Median (IQR)	P‡
TFV-DP concentrations (fmol/punch)								
Composite	66	706 (375–1023)	15.5 (−232.00 to 142.50)	0.860	47	806 (414–1265)	47.9 (−152.75 to 725.50)	0.367
PTB	45	705 (397–975)	−10.5 (−196.00 to 89.50)	0.934	26	808 (422–1208)	71.2 (−131.00 to 291.00)	0.724
EID	27	475 (83–975)	−75.8 (−594.50 to 89.50)	0.480	16	458 (144–1000)	418.6 (−119.00 to 981.50)	0.378
SB	18	753 (104–1183)	120.9 (26.40 to 806.75)	0.378	18	740 (273–1265)	6.5 (−354.00 to 726.50)	0.936
FTC-TP concentrations (pmol/punch)								
Composite	66	0.27 (0.05–0.36)	0.01 (−0.08 to 0.15)	0.934	47	0.29 (0.05–0.40)	0.00 (−0.08 to 0.13)	0.879
PTB	45	0.27 (0.05–0.36)	0.00 (−0.19 to 0.06)	0.262	26	0.30 (0.15–0.42)	0.00 (−0.07 to 0.10)	1.000
EID	27	0.16 (0.05–0.25)	0.00 (−0.08 to 0.15)	0.929	16	0.16 (0.05–0.35)	0.00 (−0.10 to 0.20)	1.000
SB	18	0.27 (0.05–0.36)	0.15 (0.01 to 0.26)	0.054	18	0.24 (0.05–0.36)	0.06 (0.00 to 0.13)	0.807

*Overall (cases and controls) DBS concentrations of TFV-DP (fmol/punch) or FTC-TP (pmol/punch), respectively; TFV-DP values below the LLOQ were set to 12.5 fmol/punch (1/2 the LLOQ); FTC-TP values below the LLOQ were set to 0.05 pmol/punch (1/2 the LLOQ).

†Difference in drug concentrations between the cases and the average concentration of their controls at weeks 4 and 8.

‡P values from the Wilcoxon signed rank test that compares the median drug concentration for cases and the average of their controls, respectively.

n, sample size.

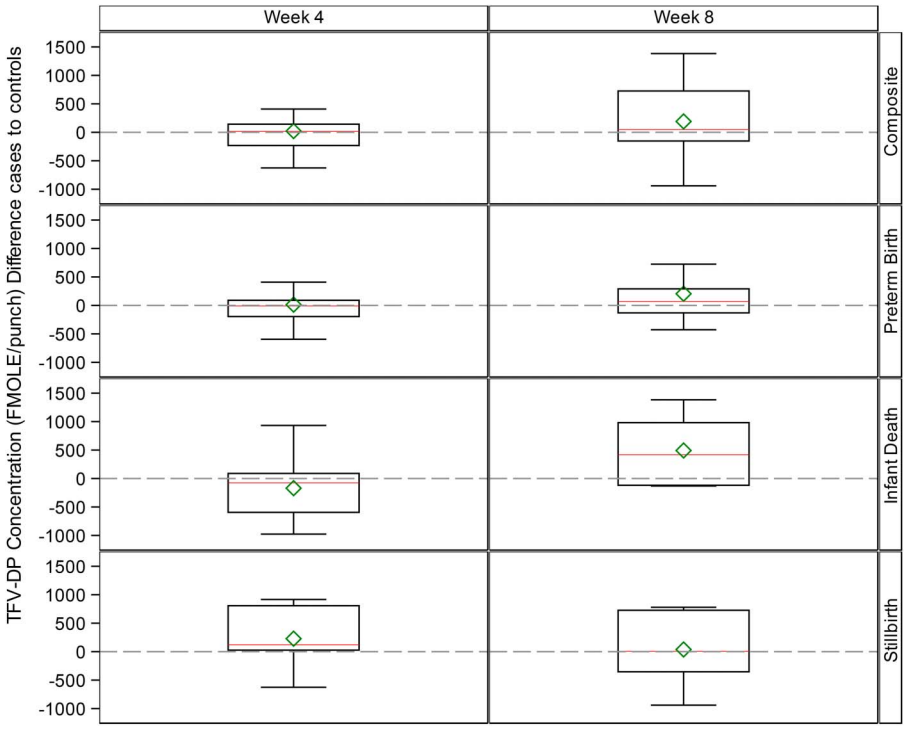

FIGURE 2. Boxplot of TFV-DP differences between cases and controls at weeks 4 and 8 post ART initiation. The red line represents the median, the green diamond represents the mean, the box margins represent the first and third quartiles, and the whiskers at the ends of the box represent 1.5× the IQR. full color online

difference between the median FTC-TP concentrations between the cases and controls.

DISCUSSION

Pregnant women in the PROMISE trial randomized to TDF-based ART had higher rates of severe adverse pregnancy/neonatal outcomes and increased risk of EID and vPTD compared with women in the ZDV-based ART arm. This follow-up case–control analysis of PROMISE study women who used TDF-based ART during pregnancy report substantial DBS concentrations of TFV-DP and FTC-TP at 4- and 8-weeks post-ART initiation among cases with severe adverse outcomes of vPTD, SB, or EID and matched controls. TFV-DP concentrations were not significantly associated with the individual outcomes of vPTD, SB, or EID or the composite severe pregnancy/neonatal outcome (vPTD, SB, or EID).

There is limited literature on TFV-DP and FTC-TP DBS concentrations during pregnancy. To the best of our knowledge, this is the first study to report intracellular TFV-DP and FTC-TP concentrations in DBS among HIV-infected pregnant women on ART. TFV-DP and FTC-TP concentrations were measured in stored DBS samples collected at 2 antepartum time points— weeks 4 and 8 after ART initiation in the PROMISE study. Pyra et al[15] reported TFV-DP concentrations in DBS among HIV-uninfected pregnant women on TDF 300 mg/FTC for PrEP and found approximately 30% lower TFV-DP concentrations in DBS in comparison to nonpregnant women after controlling for adherence. Median TFV-DP concentrations in the PROMISE study [706 (IQR, 375–1023) fmol/punch at week 4 and 808 (IQR: 414–1265) fmol/punch at week 8] are lower than previous reports with daily TDF/FTC dosing and were comparable to DBS concentrations measured with 4 TDF/FTC doses/week in HIV-negative individuals.[13] A pharmacokinetic study of TDF/FTC given under directly observed therapy in HIV-uninfected adult men and nonpregnant women reported median TFV-DP concentrations of 1030 fmol/punch at week 4 and 1464 fmol/punch at week 8.[13] A separate study in persons living with HIV on boosted TDF 300 mg reported geometric mean concentrations of 1900 fmol/punch among suppressed participants and 1100 fmol/punch among viremic individuals.[19] Maternal self-reported adherence rates were >95% in the PROMISE trial.[5] Self-report as a measure of ARV drug adherence among HIV-infected individuals has been associated with high variability and overestimation.[28,29] TFV-DP concentrations in DBS are considered an objective and reliable measure of long-term cumulative adherence,[13,14,30] whereas FTC-TP concentrations in DBS are a marker of recent dosing in both persons living with HIV and HIV-uninfected individuals.[31,32]

There are multiple factors that could affect FTC-TP and TFV-DP concentrations during pregnancy. The lower and variable TFV-DP concentrations observed in this study may have been due in part to physiological changes during pregnancy that may impact TFV-DP concentrations including alterations in absorption, distribution, and renal clearance. These changes have been observed previously among both HIV-infected women on ART and HIV-uninfected women on PrEP, respectively.[15,33] Several other physiologic changes during pregnancy may also alter DBS concentrations of these moieties, such as increased plasma volume that exceeds the percent increase in circulating red blood cells, decreased

TABLE 2. OR Estimates for Adverse Pregnancy Outcomes With TFV-DP Concentrations as Predictor*

	Week 4		Week 8	
Outcome	**Size (n)**	**OR (95% CI)**	**Size (n)**	**OR (95% CI)**
Composite safety end point	22	1.27 (0.74 to 2.18)	16	1.74 (0.66 to 4.60)
PTB	15	1.10 (0.47 to 2.53)	9	0.96 (0.28 to 3.30)
EID	9	0.91 (0.44 to 1.87)	6	1.47 (0.42 to 5.11)
SB	6	3.71 (0.36 to 38.13)	6	2.21 (0.41 to 11.90)

*Primary analysis (TFV-DP values below the LLOQ were set to 1/2 the LLOQ or 12.5 fmol/punch; and PK concentrations were natural log transformed).

hemoglobin and hematocrit production throughout the third trimester, and shortened red blood cell life span.[34,35] These collectively could contribute to lower TFV-DP and FTC-TP concentrations during pregnancy in comparison to nonpregnant adults despite similar adherence levels. The previous study by Pyra et al[15] measured high variability across all 3 trimesters pregnancy, and thus, sources of variability during pregnancy in HIV-infected and HIV-negative women should be examined further.[15] Also, PROMISE trial participants were predominantly black, which has been associated with lower TFV-DP concentrations.[19] Approximately 30% of the DBS samples analyzed were below the LLOQ for FTC-TP, compared with 6% for TFV-DP concentrations. The reasons for these observations are unclear. FTC-TP has a half-life of about 38 hours in DBS and thus serves as a measure of recent drug dosing.[32] A lack of FTC-TP in DBS samples could thus reflect intermittent medication adherence in the context of higher TFV-DP concentrations or poor medication adherence with lower TFV-DP concentrations. There are no reports in the current literature linking FTC or FTC-TP and adverse pregnancy outcomes among pregnant women living with HIV. Due to these collective factors, logistic regression analyses were limited to TFV-DP concentrations in association with adverse pregnancy outcomes.

Another study linking adverse pregnancy/neonatal outcomes with antepartum use of TDF-based ART was the Surveillance Monitoring for ART Toxicities (SMARTT) cohort of the US Pediatric HIV/AIDS Cohort study, which demonstrated reduced neonatal whole-body bone mineral content among HIV-exposed uninfected infants with at least 8 weeks of maternal reported in utero exposure to TDF-based ART.[12] Other studies linking antepartum ART use with adverse pregnancy outcomes mostly implicated protease inhibitor (PI)–based ART.[36–40] Ritonavir-boosted PI (PI/r)–based ART compared with nonboosted PI regimens has been associated with PTD.[38] In the PROMISE study design, PI/r was included as part of both the TDF-based and ZDV-based ART regimens and therefore is not an explanatory factor for the differential risk of severe adverse pregnancy outcomes between the 2 ART arms.

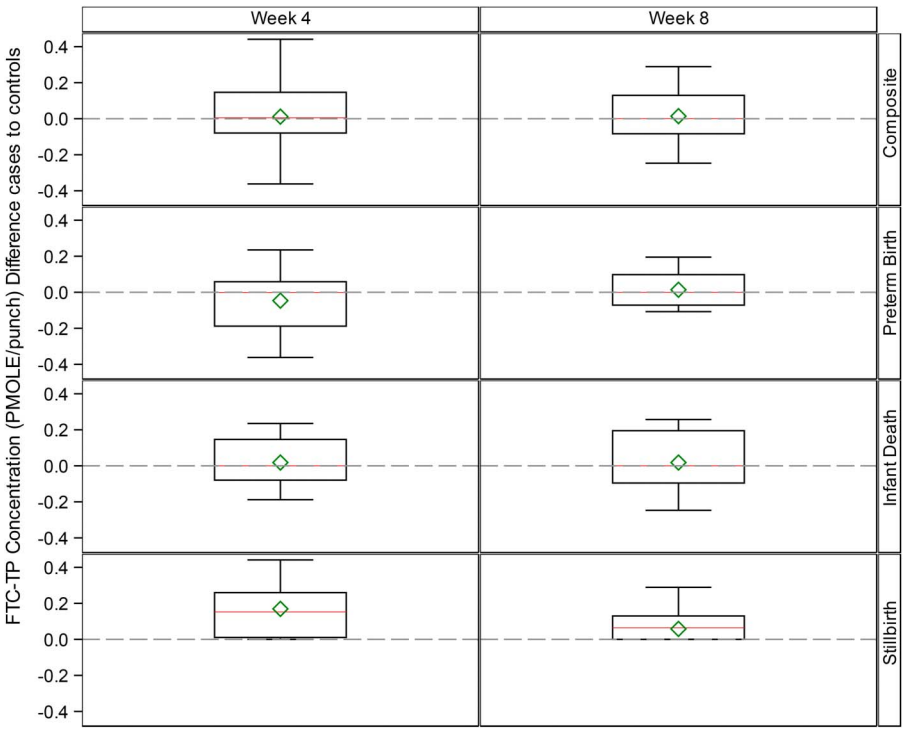

FIGURE 3. Boxplot of FTC-TP differences between cases and controls at weeks 4 and 8 post ART initiation. The red line represents the median, the green diamond represents the mean, the box margins represent the first and third quartiles, and the whiskers at the ends of the box represent 1.5× the IQR. full color online

In the PROMISE study, LPV/r doses were increased to 600/150 mg during the third trimester, consistent with product labeling. Although all the women in this study received TDF and LPV/r, our finding of similar intracellular TFV-DP concentrations among cases with severe adverse pregnancy outcomes compared with matched controls does not support our a priori hypothesis that higher intracellular TFV-DP levels would be associated with an increased risk of severe adverse outcomes. This hypothesis was based on previous studies that reported TDF and LPV/r drug–drug interactions with increased TFV in plasma and TFV-DP concentrations in peripheral blood mononuclear cells, with varying clinical implications.[8–11] Among healthy HIV-uninfected men and women, LPV/r was associated with a 32% increase in the plasma area under the concentration–time curve of tenofovir when given as TDF[8]; in a study of HIV-infected men and women, tenofovir area under the concentration–time curve was 50% higher in those on LPV/r compared with nevirapine.[9] Likewise, tenofovir renal clearance was approximately 17% lower in HIV-infected patients on LPV/r compared with those not receiving a PI.[10] In another study among HIV-infected men and women, concomitant TDF and PI/r use was associated with higher rates of creatinine clearance decline (−13.9 mL/min/yr) compared with a nonnucleoside reverse-transcriptase inhibitor–based ART regimen (−6.2 mL/min/yr) and increased serum creatinine values, over 48 weeks, respectively.[11] However, none of these prior pharmacokinetic studies involved pregnant women living with HIV as evaluated in this PROMISE subgroup analyses. Higher plasma TFV exposures have been associated with a higher risk of renal toxicity across multiple studies in nonpregnant adults,[41,42] and thus, it is possible that by examining relationships between parent TFV exposures in plasma and adverse pregnancy outcomes may have yielded different results. TFV-DP in DBS demonstrates linear relationships with plasma TFV levels and thus still provides insight into parent TFV exposures in this population.[43]

Study limitations include a small sample size that may have resulted in a type-2 error (undetected true association between TFV-DP concentration and adverse pregnancy outcomes) and lower precision. Also, sample collection time points at weeks 4 and 8 were probably not at steady state, which takes about 12 weeks in DBS in nonpregnant adults. In addition, for the analyses on FTC-TP, there were a considerable proportion of values below the LLOQ, which had to be imputed. However, there were also considerable study design strengths. Study procedures were standardized across the well-monitored multinational sites; the study ART drug protocols and stored DBS samples collected at multiple longitudinal study visits provided a unique and rare opportunity to retrospectively assess the relationship between intracellular TFV-DP concentrations and adverse pregnancy outcomes. Availability of DBS samples enabled us to conduct more objective intracellular ARV drug quantification and long-term ART adherence measures compared with other commonly used pharmacological measures such as plasma ARV drug concentrations, which are associated with short half-lives and may be subjective if study participants improve their adherence before study visits. The relative ease in the collection and storage of DBS samples and scale of use in this very large trial, and the objectivity and reproducibility of DBS measures of TFV-DP reported previously, has potential for large-scale implementation in HIV care.

CONCLUSIONS

Based on data from case–controls matched by maternal gestational age and geographical setting, maternal TFV-DP levels in DBS were not significantly associated with severe adverse outcomes (vPTD, SB, or EID) among pregnant women on TDF-based ART in the PROMISE trial. These observations did not contradict the current WHO guidance to use TDF-based regimens during pregnancy, as part of ART among HIV-infected women or TDF-based PrEP among HIV-uninfected women. Tenofovir is the backbone of both public health strategies, which are currently being rolled out in sub-Saharan Africa and India, where the PROMISE trial was conducted. However, because of the limited sample size, the possibility of associations between TFV use and severe adverse pregnancy outcomes remains. Findings from this substudy do not provide a pharmacologic explanation of the differences in severe adverse birth outcomes reported in the PROMISE trial. However, caution should be exercised in interpreting these research findings given the small sample size. Further investigation through larger scale studies and exploring other potential biologic mechanisms for the vPTD and associated SB and early infant death findings in the PROMISE study are warranted.

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