Safety of oral tenofovir disoproxil fumarate-based pre-exposure prophylaxis for HIV prevention: prospective studies in HIV-uninfected men and women

Kenneth K. Mugwanya

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Reading Committee:
Jared Baeten, Chair
Grace John-Stewart
Andreas Stergachis

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Abstract

Safety of oral tenofovir disoproxil fumarate-based pre-exposure prophylaxis for HIV prevention: prospective studies in HIV-uninfected men and women

Kenneth Mugwanya

Chair of the Supervisory Committee:
Jared M. Baeten, Vice Chair, Department of Global Health,
Professor, Departments of Global Health, Medicine, and Epidemiology

Antiretroviral pre-exposure prophylaxis (PrEP) with tenofovir disoproxil fumarate (TDF) alone or when co-formulated with emtricitabine (FTC), the same medication used for treatment of HIV infection, is a recommended and highly effective strategy to reduce the risk of sexual acquisition of HIV.

The central objective of the studies described in this dissertation was to quantify the risk of potential off-target safety signals associated with TDF-based PrEP use in HIV-uninfected men and women with the overarching goal of providing the evidence base for clinical practice guidelines to accelerate population level delivery of PrEP to fight the global HIV epidemic. The specific aims include to: 1) determine whether TDF-based PrEP causes clinically significant decline in glomerular filtration rate (eGFR), a commonly used-measure of overall kidney function, in HIV-uninfected men and women; 2) determine whether TDF-based PrEP causes proximal tubular dysfunction when used as PrEP and whether proximal tubular dysfunction is associated with clinically relevant decline eGFR; 3) quantify infant exposure to tenofovir and emtricitabine via maternal

breast milk when used as PrEP by lactating HIV-uninfected women; 4) determine whether open-label PrEP use for HIV prevention is associated with reduction in safer sex practices (i.e., sexual risk compensation); and 5) review and summarize the totality of empirical literature on the TDF-induced off-target effects when use as PrEP.

Findings: *Effect of TDF-based PrEP on eGFR*: In a large randomized, placebo-controlled trial of daily oral TDF and FTC-TDF PrEP among 4640 heterosexual persons, with median per-protocol follow-up of 18 months and maximum follow-up of 36 months, PrEP resulted in a small but non-progressive decline in eGFR that was not accompanied by a substantial increase in the risk of clinically relevant (≥25%) eGFR decline. The decline quickly resolves within weeks after TDF discontinuation. *Effect of FTC-TDF PrEP on proximal tubular dysfunction:* In a randomized, placebo-controlled comparison among >1500 HIV-uninfected men and women, FTC-TDF PrEP was not associated with increased risk for proximal tubular dysfunction up to 24 months nor was proximal tubular dysfunction associated with clinically relevant decline in eGFR. *Infant exposure to PrEP via breastfeeding*: Among lactating women using FTC-TDF PrEP during early postpartum, the estimated infant doses received from breastfeeding and the resultant infant plasma concentrations for both tenofovir and emtricitabine are 12500- and >200-fold below the respective proposed pediatric doses used for therapeutic treatment of infant HIV infection and for prevention of infant postnatal HIV infection and tenofovir was unquantifiable in a majority of infant plasma samples, suggesting that PrEP can be safely used during breastfeeding with minimal infant drug exposure.

Sexual risk compensation: The transition from a double-blinded, placebo-controlled phase to one in which all participants were aware that they were receiving active, effective PrEP in the Partners PrEP Study, provided a natural experiment to assess behavioral risk compensation. PrEP given as part of a comprehensive HIV prevention package, did not result in substantial changes in risk-taking sexual behavior by heterosexual couples.

Summary of current empirical literature: TDF-based PrEP is generally safe and well tolerated in HIV-uninfected men and women, and infant exposure via breastfeeding is minimal. The risk of the small, non-progressive, and reversible decline in eGFR and bone mineral density as well as the potential for selection of drug resistant viral mutation associated with PrEP are outweighed, at the population level and broadly for individuals, by PrEP's substantial reduction in the risk of HIV infection. These data support the safety of TDF-based PrEP for prevention HIV combination with safer sex practices

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DEDICATION

To my Angel Kimberly and my confidant Keith.

To my mom (RIP) and dad (RIP), I hope I made you proud!

Introduction

Introduction

This dissertation addresses key unanswered questions related to the safety of tenofovir disoproxil fumarate (TDF)-based pre-exposure prophylaxis (PrEP) in HIV-uninfected persons including kidney toxicity, infant exposure to PrEP medications via breast milk, and potential sexual risk compensation. The studies reported here provide comprehensive investigation and synthesis of PrEP-related off-target effects in HIV-uninfected men and women and provide novel empirical evidence to guide policy and clinical practice decision making with the overarching goal of accelerating population-level PrEP implementation to reduce HIV acquisition risk.

Daily oral PrEP with TDF alone or when co-formulated with emtricitabine (FTC) is a powerful and highly effective approach for prevention against HIV acquisition when taken with sufficient adherence. Several proof-of-concept randomized, placebo-controlled clinical trials conducted in diverse geographies and at-risk populations demonstrated HIV protective effectiveness ranging between 44-75% in randomized comparisons and >90% in persons adherent to PrEP as prescribed.[1-4] Subsequent follow-on open-label extensions and pragmatic studies have provided further confirmation of high protective effectiveness for PrEP against HIV infection in “real world” settings.[5-7]

In July 2012, after reviewing the available clinical trial results, the U.S. Food and Drug Administration approved a prevention indication for the use of FTC-TDF in combination with safer sex practices for PrEP to reduce the risk of sexually acquired HIV in adults at

high risk.[8] Subsequently, several normative organizations including the U.S. Centers for Diseases Control and Prevention and the World Health Organization issued detailed practice guidelines for use of PrEP as a prevention strategy in persons at substantial risk of HIV infection.[9,10] Recent approvals by drug authorities in several countries in Europe, Africa, and South America will further accelerate wide-scale use of PrEP in these regions.[11,12]

FTC and TDF are widely prescribed as part of combination antiretroviral therapy for the treatment of HIV.[10] While generally safe and well tolerated in HIV-infected persons, TDF use is associated with increased risk of some toxicities, including renal impairment and loss of bone mineral density.[13,14] Moreover, use of antiretroviral medications, for treatment of HIV and potentially for prophylaxis as well, carries some risk of selection for HIV viruses harboring antiretroviral resistance. Importantly, all biomedical prevention interventions including PrEP require extraordinary safety standard compared to when used for therapeutic treatment, as persons using the preventative intervention are generally healthy and have only a chance of contracting the condition being prevented. Thus, data to inform the safety of TDF and FTC-TDF as PrEP for HIV prevention are essential. In proof-of-concept efficacy trials,[1-4,15,16] there were no overt PrEP-related adverse effects in intent-to-treat analyses comparing PrEP to placebo. However, drug exposure as measured by tenofovir concentration in plasma, an important determinant of drug toxicity, was inadequate in some of the reported PrEP studies and such lack of evidence for potential adverse effects in these populations may not necessarily imply null safety PrEP-effect. Moreover, intention-to-treat analyses in placebo-controlled

randomized clinical trials underestimate the treatment effect in presence of non-compliance and such are ideal for investigation when the primary question of interest is safety (i.e., anticonservative for safety-related questions). Thus, empirical studies that are designed to specifically quantify the risk of PrEP off-target effects outcomes are important to address critical unanswered safety questions for population-level delivery of PrEP to provide the evidence base to guide policy and clinical decision making. These studies help to anticipate the risk of toxicity with prolonged use of FTC-TDF in healthy adults and have the potential to promote biomarker identification to accelerate future drug development.

Effect of emtricitabine-tenofovir disoproxil fumarate pre-exposure prophylaxis on glomerular kidney function among HIV-uninfected men and women: A randomized, placebo-controlled trial

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Does TDF-based PrEP cause clinically relevant declines in glomerular filtration rate (GFR) in healthy HIV-uninfected men and women? TDF when used as part of combination antiretroviral therapy for the treatment of HIV is infrequently associated with renal impairment including decline in estimated GFR, a commonly-used measure of overall kidney function, ranging from mild to occasionally severe.[13] Extrapolating results from HIV-infected populations to the PrEP context, however, is potentially confounded by HIV infection, concomitant use of other antiretroviral medications as well as other co-morbidities. In first generation PrEP clinical trials, PrEP exposure was not associated with overt kidney toxicity based on change in serum creatinine from baseline values but graded creatinine events were more frequent in the active PrEP arms in all trials, although the differences did not reach statistical significance.[2-4,15,16] Whether TDF

exposure among HIV-uninfected adults causes more subtle, but still clinically relevant declines in eGFR is of considerable importance particularly in populations with high adherence to PrEP. In chapter 2, we present a large randomized placebo-controlled study among HIV-uninfected African men and women, to determine whether TDF-based PrEP causes clinically relevant decline in eGFR in HIV-uninfected adults. We leveraged unique resources in the Partners PrEP Study, a large randomized, placebo-controlled trial of daily oral TDF and FTC-TDF PrEP among African heterosexual HIV-uninfected members of serodiscordant couples, including availability of banked specimens, regular safety monitoring in both men and women across abroad range of age groups. Adherence as measured by tenofovir concentration in plasma in the Partners PrEP Study was the highest of any published PrEP clinical trial making our study cohort an ideal population to study TDF-related kidney toxicities in healthy HIV-uninfected persons. The primary analysis for this dissertation aim was a per-protocol safety analysis, which enriched for drug exposure in the primary analysis. To assess study end points, we used standard regression methods as the primary approach and marginal structural models weighted with inverse probability of censoring weights in sensitivity analyses that accounted for drug discontinuation and potential selection bias.

Effect of emtricitabine-tenofovir disoproxil fumarate pre-exposure prophylaxis on the risk of proximal tubular dysfunction among HIV-uninfected men and women.

Is FTC-TDF PrEP associated with subclinical proximal tubular dysfunction? The proximal tubule epithelial cells are the primary site for TDF-based off-target effects resulting in impairment of tubular solute transportation ranging from mild to severe

dysfunction. Clinically significant proximal tubular dysfunction, or Fanconi syndrome, is a rare but serious complication characterized by generalized low molecular-weight proteinuria, euglycemic glycosuria, hypophosphatemia, phosphaturia, metabolic acidosis, and hypouricemia. Loss of body solutes particularly if it involves chronic loss of phosphorus could lead to functional vitamin D deficiency with consequent loss of bone mineral density. No study has assessed the effect of TDF-based PrEP on proximal tubular function in HIV-uninfected women, particularly those with high drug exposure. In Chapter 3 using archived urine and serum samples in the Partners PrEP Study, we investigated, in a randomized placebo-controlled comparison, whether FTC-TDF causes tubular dysfunction when used for PrEP and whether tubular dysfunction is associated with subsequent clinically relevant decline (≥25%) eGFR. These data address important pending questions regarding early kidney tubular dysfunction that can occur in absence of full manifestation of glomerular dysfunction and will provide guidance on the utility of route tubular function testing in persons using PrEP.

Pre-exposure prophylaxis use by breastfeeding HIV-uninfected women: a short-term study of antiretroviral excretion in breastmilk and infant absorption.

Is tenofovir and emtricitabine transmitted in clinically significant concentrations to nursing infants through breast milk when as PrEP by HIV-uninfected women?

Pregnancy and early postpartum breastfeeding represent periods of heightened HIV risk for women.[17] Moreover, incident maternal infections during breastfeeding might increase mother-to-child HIV transmission as acute HIV infection is associated with high HIV viremia.[18-20] Daily oral PrEP offers an effective female-controlled option to reduce

the risk of sexual HIV acquisition for women who are pregnant or breastfeeding, with the advantage relative to other prevention current methods that it does not require cooperation of sexual partners. As PrEP becomes more widely used in heterosexual populations, women who are breastfeeding may be prescribed PrEP and an important consideration is its safety in infants who are breastfed by women taking PrEP. Decisions about the safety of breastfeeding during maternal ingestion of drugs require knowledge of the amount of drug which might be present in breast milk. No published data on infant exposure and safety with PrEP exposure through breastfeeding. Pregnant and breastfeeding women were excluded in PrEP trials, and those who became pregnant stopped study drug. Experience with tenofovir and emtricitabine pharmacokinetics in pregnancy and postpartum breast feeding outside of clinical trials has largely been among HIV infected women for prevention of maternal to child transmission but because a majority of these studies infants were exposed to circulating drugs via the placenta and in addition to oral TDF,[21-23] their pharmacokinetic parameters likely represent a combination of both vertical- and oral-administration pharmacokinetic patterns. In chapter 4, we report a short-term, prospective, open-label, pharmacokinetic study of daily oral FTC-TDF PrEP among African HIV-uninfected lactating women-infant pairs to investigate the transfer of tenofovir and emtricitabine into breast milk and subsequent infant exposure via breastfeeding when used as PrEP by lactating women; this study is a first in the field. Data garnered here provide critical empirical evidence base for risk-to-benefits balance assessment of initiating or continuing maternal FTC-TDF PrEP use during lactation.

Sexual behavior of heterosexual men and women receiving antiretroviral pre-exposure prophylaxis for HIV prevention. A longitudinal analysis

Do persons using open-label PrEP for HIV prevention engage in reduced safer-sex behaviors? Evidence for the effectiveness of new HIV-prevention strategies, including PrEP has spurred optimism that the global HIV epidemic might be reversed. However, important questions of whether HIV-negative partners who know they are protected by prophylaxis would compensate for this by increasing their sexual risk-taking such as through increasing their levels of condomless sex acts have been echoed.[24,25] This phenomenon commonly referred to as risk compensation, could theoretically off-set the protective benefits of PrEP as well as increase the risk for other complications including sexually transmitted infections and unintended pregnancy. In chapter 5, we conducted a longitudinal analysis to investigate whether use of open-label PrEP in HIV-uninfected men and women in HIV-serodiscordant couples resulted in reduced safer sex practices (i.e. risk compensation). We leveraged on the unique opportunity of the open-label extension of the Partners PrEP Study among serodiscordant heterosexual couples, in which the placebo arm was stopped early by the study Data and Safety Monitoring Board after definitive evidence of PrEP benefit against HIV was found. HIV-uninfected participants originally randomized to receive active PrEP (i.e. either TDF or FTC-TDF) continued to take PrEP without interruption in the study procedures including monthly evaluation sexual behaviors except that participants were actively informed about the demonstrated PrEP effectiveness against HIV. This transition from a blinded, placebo-controlled phase to an open-label extension in which all participants aware they were

receiving active, efficacious PrEP provided a “natural experiment” to evaluate potential behavioral risk compensation.

A systematic review of the safety of oral tenofovir disoproxil fumarate-based pre-exposure prophylaxis for HIV prevention.

Use of antiretroviral medications for prophylaxis, like all other biomedical interventions carries some risk of off-target effects that must be balance against the powerful HIV protective benefits accorded by PrEP. In Chapters 6, we conducted a comprehensive review and synthesis of the totality of current literature on the safety of TDF-based PrEP, with focus on tolerability, kidney function, bone density, HIV resistance, sexual and reproductive health. This central goal of the review was to summarize and weigh the risks of PrEP in context of its protective effectiveness against HIV. Further, we discuss potential alternative PrEP drugs and formulations that are currently being evaluated including other oral agents, intravaginal rings, and longer-acting injectable agents.

Effect of emtricitabine-tenofovir disoproxil fumarate pre-exposure prophylaxis on glomerular kidney function among HIV- uninfected men and women: a randomized placebo-controlled trial

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Effect of Emtricitabine-Tenofovir Disoproxil Fumarate Pre-exposure Prophylaxis on Glomerular Kidney Function among HIV-Uninfected Men and Women: A Randomized Placebo-controlled Trial

Kenneth K. Mugwanya, MBChB, MS [a,b]; Christina Wyatt, MD, MS[c]; Connie Celum, MD, MPH[a,d,e]; Deborah Donnell, PhD[d,f]; Nelly R. Mugo, MBChB, MPH[d,g]; Jordan Tappero, MD, MPH[h]; James Kiarie, MBChB, MPH[i,d]; Allan Ronald, MD[j]; Jared M. Baeten, MD, PhD[a,d,e]; for the Partners PrEP Study Team*

From the Departments of [a]Epidemiology, [d]Global Health, and [e] Medicine, University of Washington, Seattle, USA; [b]Division of Disease Control, School of Public Health, Makerere University, Kampala, Uganda; [c]Department of Medicine, Division of Nephrology, Mount Sinai School of Medicine, New York, USA; [f]Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, USA; [g] Kenya Medical Research Institute, Nairobi, Kenya; [h]Division of Global Health Protection, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, USA; [i] Department of Obstetrics and Gynecology, University of Nairobi, Nairobi, Kenya; [j]Department of Medicine, University of Manitoba, Winnipeg, Canada; * Members of the Partners PrEP Study Team listed in the Acknowledgements section

Short title: PrEP and Decreased Glomerular Filtration Rate

Correspondence to: Jared Baeten, MD, PhD University of Washington 325 Ninth Avenue UW Box 359927 Seattle, WA 98104 Phone: (206) 520-3808 Fax: (206) 520-3831

Email: jbaeten@uw.edu

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Abstract

Importance: Tenofovir disoproxil fumarate (TDF) use has been associated with declines in the estimated glomerular filtration rate (eGFR) when used as part of antiretroviral treatment by HIV infected persons, but limited data are available for risk when used as pre-exposure prophylaxis (PrEP) for HIV prevention.

Objective: To determine whether TDF-based PrEP causes eGFR decline in HIV uninfected adults.

Design, Setting, and Participants: A per-protocol safety analysis of changes in eGFR in the Partners PrEP Study, a randomized, placebo-controlled trial of daily oral TDF and emtricitabine (FTC)-TDF PrEP among African heterosexual HIV-uninfected members of serodiscordant couples conducted from 2008 to 2012.

Main Outcomes and Measures: Pre-defined outcomes of this analysis were mean eGFR change and a ≥25% eGFR decline from baseline. eGFR was calculated using **Chronic Kidney Disease Epidemiology Collaboration.**

Results: Of 4640 subjects randomized and followed on either once daily TDF (n=1548), FTC-TDF (n=1545), or placebo (n=1547), 63% were male. At enrollment, median age was 35 years (range 18-64) and mean eGFR was 130 mL/min/1.73m^2. During a median follow-up of 18 months (interquartile range 12-27), mean within-group eGFR change from baseline was +0.14 mL/min/1.73m^2 for TDF, -0.22 mL/min/1.73m^2 for FTC-TDF, and +1.37 mL/min/1.73m^2 for placebo, translating into average declines in eGFR attributable to PrEP versus placebo of -1.23 mL/min/1.73m^2 (95% CI -2.06, -0.40; p=0.004) for TDF and -1.59 mL/min/1.73m^2 (95% CI -2.44, -0.74; p<0.001) for FTC-TDF. The difference in mean eGFR between PrEP and placebo appeared by one month

after randomization, was stable through twelve months, and then appeared to wane thereafter. The proportion of persons who developed a confirmed ≥25% eGFR decline from baseline by 12 and 24 months was 1.3% and 1.8% for TDF and 1.2% and 2.5% for FTC-TDF, and these frequencies were not statistically different compared to placebo (0.9% and 1.3% by 12 and 24 months).

Conclusion and Relevance: In this large randomized, placebo-controlled trial among heterosexual persons, with median follow-up of 18 months and maximum follow-up of 36 months, daily oral TDF-based PrEP resulted in a small but non-progressive decline in eGFR that was not accompanied by a substantial increase in the risk of clinically relevant (≥25%) eGFR decline.

Trial Registration: Clinicaltrials.gov Identifier: NCT00557245

Introduction

Antiretroviral pre-exposure prophylaxis (PrEP) with tenofovir disoproxil fumarate (TDF) alone or in combination with emtricitabine (FTC-TDF) has demonstrated protection against HIV acquisition in diverse geographical and at-risk populations[1-4], with effectiveness of 44-75% in randomized, placebo-controlled comparisons and ~90% in subset analyses of adherent participants.

Among HIV-infected individuals receiving antiretroviral therapy, studies have consistently demonstrated a significantly higher frequency of kidney dysfunction, including decline in estimated glomerular filtration rate (eGFR), in patients receiving TDF-containing regimens compared to those receiving regimens not containing TDF.[13,26-29] Extrapolating results from these studies to the PrEP context, however, is potentially confounded by HIV infection and concomitant use of other antiretroviral medications. In PrEP clinical trials,[1-4,16] PrEP exposure was not associated with overt kidney toxicity. However, whether TDF exposure among HIV-uninfected adults causes more subtle but still clinically relevant declines in eGFR requires exploration. Use of PrEP with FTC-TDF is now recommended by the US Centers for Disease Control and Prevention and the World Health Organization,[9,10] lending greater importance to profiling the safety signals of FTC-TDF in HIV-uninfected persons.

We investigated the effect of daily oral TDF-based PrEP on eGFR in HIV-uninfected adults in a placebo-controlled trial of PrEP in which PrEP adherence was high.

Methods

Study design and participants

Data were from the Partners PrEP Study,[1,30] a phase III, randomized, placebo-controlled trial of daily oral TDF and FTC-TDF PrEP among heterosexual HIV-uninfected members of HIV serodiscordant couples (Clinicaltrials.gov number NCT00557245). Between July 2008 and November 2010, 4747 HIV serodiscordant heterosexual couples were enrolled at nine research sites in Kenya and Uganda. Eligible HIV-uninfected participants were ≥18 years of age, did not have active hepatitis B infection, were sexually active, were not pregnant or breastfeeding, had normal renal function (defined by serum creatinine ≤1.3 mg/dL for men / ≤1.1 mg/dL for women and Cockcroft-Gault calculated creatinine clearance of ≥60 mL/min), not receiving ongoing therapy with agents with known significant nephrotoxic potential, and did not have diabetes requiring hypoglycemic medication or active and clinically significant cardiac disease. HIV-uninfected partners were randomly assigned in a 1:1:1 ratio to one of the three study groups: TDF, FTC-TDF, or an inert placebo. TDF and FTC were dosed at 300 mg daily and 200 mg daily, respectively; these doses are also the standard for treatment of HIV.[31]

HIV-uninfected partners were followed monthly up to 36 months with HIV testing, study medication refill for 30 days, collection of the prior month's unused medication, and adherence counseling. Adherence to study medication was assessed by pill counts of returned bottles at each monthly visit. Laboratory safety, including serum creatinine, was evaluated at baseline, month 1 and quarterly thereafter. Grading of adverse events

was based on the 2009 DAIDS grading systems adapted to local laboratory reference ranges.[32] Study medication was permanently discontinued in subjects who experienced HIV acquisition and was withheld in women who became pregnant for the duration of pregnancy and breastfeeding. Additionally, study medication was temporarily withheld if a participant had a confirmed creatinine abnormality (i.e., confirmed with repeat testing, ideally completed within 7 days) defined as serum creatinine increase 1.1 times upper limit of normal and or >1.5-fold change from baseline. Study drug could be restarted if serum creatinine returned to normal or within 1.3-fold of the baseline value. Study drug was permanently discontinued with a confirmed ≥grade 2 creatinine abnormality (defined as ≥1.4 times the upper limit of normal or a Cockcroft-Gault calculated creatinine clearance <50 mL/min).

The study protocol was approved by the University of Washington Human Subjects Review Committee and ethics review committees at each of the study sites. All participants provided written informed consent. Study progress was reviewed by an independent Data and Safety Monitoring Board (DSMB); in July 2011 the DSMB recommended that the placebo arm be discontinued, due to definitive demonstration of PrEP efficacy against HIV acquisition. Additionally, the DSMB recommended continued blinded follow-up of the active arms to garner additional data on safety and efficacy of FTC-TDF vs TDF.[33]

Assessment of GFR

The eGFR was calculated from serum creatinine using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Equation.[34] The CKD-EPI equation has recently

been validated in African populations and provides more accurate estimates for eGFR values in the normal range than both the Modification of Diet in Renal Disease Study and Cockoft-Gault equations when compared to a direct measure of GFR by iohexol clearance.[35,36] Serum creatinine was measured at baseline, month 1, and quarterly thereafter. For this analysis, pre-defined study outcomes were mean eGFR change from baseline and a decline in eGFR of ≥25% compared to baseline, as confirmed by a second measurement obtained prior to study drug discontinuation. The cutoff of ≥25% decline in eGFR was adapted from established criteria for the diagnosis of acute kidney injury[37]; eGFR decline of this magnitude has been associated with increased morbidity and mortality.[38-40] eGFR values >200 were imputed to 200 mL/min/1.73m^2 consistent with the range of GFR values in the CKD-EPI study.[34] All site laboratories participated in regular proficiency testing.

Statistical analysis

The primary analysis was a per-protocol safety analysis, censoring participants' visits occurring after >4 consecutive weeks off study drug for any reason (including protocol required safety hold, missed visits, and HIV seroconversion). Our aim was to estimate the effect of continuous PrEP use on eGFR; recognizing that full adherence is naturally impractical in clinical settings, the per-protocol analysis is a robust approach to address drug safety.[41] The primary analyses were conducted using data collected from November 2008 through July 2011, when the placebo arm of the trial was discontinued.

To assess the study endpoints (absolute mean eGFR difference from baseline and time to first confirmed ≥25% eGFR decline from baseline), we used standard regression methods as the primary approach, and marginal structural models weighted with inverse probability of censoring weights in sensitivity analyses.[42,43] Marginal structural models have been proposed as one method to address potential selection bias or confounding that can result from post-randomization nonadherence and censoring.[42,43] We also evaluated treatment effects among subgroups of sex and age. For all analyses, each active PrEP arm (TDF and FTC-TDF) was compared separately to placebo; additional testing compared TDF versus FTC-TDF. The net mean eGFR difference associated with PrEP was computed as the difference in mean eGFR change from baseline between the TDF or FTC/TDF groups and the placebo group. Outcome and person-time were evaluated at 1 month and then quarterly.

For the absolute mean change in eGFR, linear regression and marginal structural linear models were fit using generalized estimating equations, with time since enrollment fitted using 3 knot restricted cubic spline (details provided in appendix). Models were adjusted for site, sex, and age with an independent correlation structure and robust standard errors to correct for the within-person correlations.[44] Treatment effects by visit-month since randomization were generated in a separate model fitted with treatment by categorical time interaction.

For the analysis of time to the first confirmed decline in eGFR of ≥25%, we used right-censored Kaplan-Meier methods to estimate the cumulative probability, standard Cox

proportional hazards models to estimate relative hazard rates,[45] and marginal structural Cox proportional hazards models with time-dependent inverse probability-of-censoring weights [42,43] with robust standards errors derived by the Lin and Wei variance estimator[46] to control for within-person correlation (see etable 1b for details of weight estimation). Cox proportional hazard models were adjusted for baseline eGFR as 3 knot restricted cubic spline and stratified according to site, age groups, and sex. Ties were handled using the Efron approximation method.[47,48]

Three additional sensitivity analyses were conducted: 1) a time to event analysis of the repeated events of a ≥25% eGFR decline using the Andersen-Gill counting process approach in Cox regression models under the per-protocol approach[49]; 2) an intention-to-treat analysis including all randomized persons with at least one post-randomization creatinine measurement regardless of time off study medication using data collected through July 2011; and 3) an intention-to-treat approach including the additional follow-up of the two active PrEP arms after the suspension of the placebo arm in July 2011.

Finally, in exploratory analysis, we evaluated baseline factors associated with a ≥25% decline in eGFR from baseline. Additionally, we evaluated the frequency of a >1.5 fold increase in serum creatinine above baseline and study medication discontinuation related to creatinine abnormalities. Analyses were conducted using SAS software (version 9.3, SAS Institute).

Results:

Of the 4747 HIV-uninfected individuals enrolled in the Partners PrEP Study, 4640 (98%) were included in the primary per-protocol safety analysis: 1548 in the TDF group, 1545 in the FTC-TDF group, and 1547 in the placebo group (Figure 0:1). Of 107 excluded, 51 did not have any post-randomization serum creatinine measurement and 56 were off study medication >4 consecutive weeks by their first creatinine measurement, generally due to treatment refusal, missed visits, or pregnancy. Of the 4640 participants included in the primary analysis, 63% were male and mean age at enrollment was 35 years (range 18-64). Baseline characteristics were comparable across the three treatment groups (Table 0:1). Overall, 6548.8 person-years were accrued during median follow-up of 18 months (interquartile range 12 to 27) for this per-protocol safety analysis, representing approximately 88% of the total person-years collected in the study [i.e., 12% of person-years were excluded from this per-protocol analysis due to post-randomization censoring, mostly due to missed visits (5%), pregnancy (2.5%), and treatment refusal (1.4%)].The distribution of triggers for censored person-time were no more frequent in the active PrEP arms than in the placebo group including that resulting from creatinine abnormality-related study medication hold (0.7% overall: 0.6% for TDF, 0.8% for FTC-TDF, and 0.6% for placebo; $p>0.05$ for both TDF and FTC-TDF vs placebo). Because of the truncated follow-up of the placebo group, few participants (n=718) contributed ≥30 months of follow-up in the primary per-protocol analysis. An additional 2638 person-years were accrued in the active PrEP arms after July 2011 and contribute to the sensitivity analyses using the intent-to-treat approach. Overall, including the additional follow-up of the active PrEP arms beyond July 2011 in the

sensitivity analysis, participants were followed for a median of 30 months (interquartile range 24 to 36); with the TDF and FTC-TDF arms observed for a median of 36 months (interquartile range 27 to 36).

Effect of TDF and FTC-TDF PrEP on absolute mean eGFR change from baseline

Overall, mean eGFR at baseline was 130 mL/minute/173m^2 for the TDF group, 129 mL/minute/173m^2 for the FTC-TDF group, and 129 mL/minute/173m^2 for placebo group. During randomized treatment, PrEP was associated with a small but statistically significant decline in eGFR. During a median 18 months of PrEP treatment, the mean within-group eGFR change from baseline was +0.14 mL/min/1.73m^2 for the TDF group, -0.22 mL/min/1.73m^2 for the FTC-TDF group, and +1.37 mL/min/1.73m^2 for placebo, representing absolute mean eGFR change associated with PrEP of -1.23 mL/min/1.73m^2 (95%CI -2.06, -0.40; p=0.004) for TDF and -1.59 mL/min/1.73m^2 (95%CI -2.44, -0.74; p<0.001) for FTC-TDF (Table 0:2). Compared to baseline eGFR, the estimated differences in mean eGFR change from baseline between PrEP and placebo translated into a 0.9% and 1.2% decline in eGFR that was associated with TDF and FTC-TDF, respectively. The difference between PrEP and placebo in eGFR changes from baseline appeared by 4 weeks after randomization (-1.70 mL/min/1.73m^2, p=0.001 for TDF vs. placebo and -2.42 mL/min/1.73m^2, p<0.001 for FTC-TDF vs. placebo), was stable to 12 months, and then appeared to gradually wane thereafter (at 24 months: -0.81 mL/min/1.73m^2, p=0.31 for TDF vs. placebo and -0.42 mL/min/1.73m^2, p=0.63 for FTC-TDF vs. placebo). The pattern of change over time in crude mean eGFR difference from baseline had upper limits of the 95% confidence intervals under 3

mL/min/1.73m^2 through 36 months post-randomization with the additional follow-up of the two active PrEP arms (Figure 0:2). Overall, PrEP effects were consistent among subgroups of age and gender and in all sensitivity analyses including marginal structural models.

Overall, confirmed CKD-eGFR decline to <60 mL/min/1.73m^2 was recorded in two participants, both in the TDF group. First, a 58 year-old, 61 kg male with baseline CKD-eGFR of 99 mL/min/1.73m^2 had CKD-eGFR of 10 mL/min/1.73m^2 (serum creatinine 7.2 mg/dL) at 36 months with concurrent 2+ dipstick proteinuria, grade 4 liver transaminases, and clinical features suggestive of acute hepatitis. There was no concurrent nephrotoxic medication. Study drug was permanently discontinued and eGFR returned to >60mL/min/1.73 m^2 within 4 weeks. Second, a 34 year-old, 58 kg male with baseline eGFR of 154 mL/min/1.73m^2 had CKD-eGFR of 57 mL/min/1.73m^2 (serum creatinine 1.53 mg/dL) at 30 months with history of recent relocation to a hot and dry region. Urine dipstick and liver enzymes were normal and there was no concomitant medication. Study drug was discontinued permanently and eGFR returned to >60mL/min/1.73 m^2 within 2 weeks. Both events were conservatively managed.

Effect of TDF and FTC-TDF on a ≥25% eGFR decline from baseline

Overall, confirmed ≥25% eGFR decline was rare (Table 0:3). A total of 72 events occurred in the study, 68 during the per-protocol observation period and 4 during the censored period. Of these 68 events, 23 were in the TDF group (incidence rate=1.08 per 100 person-years), 27 were in the FTC-TDF group (incidence rate=1.24 per 100

person-years), and 18 were in the placebo group (incidence rate=0.83 per 100 person-years), representing attributable incidence rate difference of 0.41 per 100 person-years (95% CI -0.19, 1.01) for FTC-TDF and 0.25 per 100 person-years (95% CI -0.33, 0.83) for TDF alone, neither of which was statistically different than placebo. The proportion of persons who developed a ≥25% eGFR decline from baseline was 1.3% for TDF, 1.2% FTC-TDF, and 0.9% for the placebo by 12 months; 1.8% for TDF, 2.5% FTC-TDF, and 1.3% for the placebo by 24 months; and 1.8% for TDF, 2.5% for FTC-TDF, and 2.2% for placebo by 36 months (Figure 0:3). Compared to placebo, the adjusted relative hazards for a confirmed ≥25% eGFR decline from baseline associated with active PrEP was 1.33 (95% CI 0.71, 2.48; p=0.37) for TDF alone and 1.45 (95% CI 0.79, 2.64; p=0.23) for FTC-TDF (Table 0:3). In exploratory analysis, older age, female gender, and higher baseline eGFR appeared to be independently associated with increased likelihood for ≥25% eGFR decline from baseline (p <0.05 for all). Overall, PrEP effects were consistent among subgroups of age and gender and in all sensitivity analyses including marginal structural models.

Frequency of a >1.5-fold serum creatinine increase above baseline

Overall, a total of 451 unconfirmed events of serum creatinine increase >1.5-fold above baseline were recorded (n=237 participants). Of these, 159 (35%) were confirmed on repeat measurement from 47 (1% of 4696 total subjects regardless of time off study medication) participants: 63 events were in the TDF group, 60 in the FTC-TDF group, and 36 in the placebo. Study medication was permanently discontinued in 5 of these

subjects per protocol specification (2 each in the TDF and FTC-TDF groups and one in placebo; all had borderline creatinine clearance at baseline range 60 to 72 mL/min).

Discussion

In this safety analysis from a large randomized placebo-controlled trial, daily oral TDF-based PrEP resulted in a small but statistically significant decrease in estimated glomerular filtration rate – specifically, a change relative to baseline <1.5%, which was non-progressive for 36 months and was not accompanied by a significant increase in the likelihood of a clinically-relevant change in eGFR (i.e., ≥25%). The observed results were consistent in different subgroups and in multiple statistical approaches to evaluate the treatment causal effects. To our knowledge, this is the largest randomized trial to quantify the magnitude of subclinical eGFR decline in the presence of high adherence to PrEP in both men and women and across a broad range of ages.

Glomerular filtration rate is easily estimated from serum creatinine using prediction equations that take into account age, sex, and race or body weight, and provides a more reliable and accurate index for detection and monitoring of glomerular kidney dysfunction compared to serum creatinine alone. Age-related decline in GFR has been considered part of the normal aging process declining by approximately 1 mL/min/1.73 m^2 per year beginning after 40 years of age.[50,51] However, the clinical significance of drug-related subclinical eGFR decrease in healthy HIV uninfected adults is unknown. In the current study, we observed small subclinical declines in mean eGFR with upper bounds of the 95% confidence intervals in the range of 1-3 mL/min/1.73m^2; PrEP effects

were reversible after drug discontinuation. Because PrEP use is a time-dependent intervention for months or years of greatest HIV risk and not life-long, the clinical significance of the observed changes in eGFR may be quite small. Early TDF-induced nephrotoxicity appears to be reversible in both HIV infected and uninfected persons after TDF discontinuation.[52,53] In our study, an increase in the within-group eGFR over-time for the placebo and TDF groups is likely a regression to the mean rather than a true biological effect[54] and the between-group differences represent unbiased estimates of PrEP effects; analysis of covariance yielded similar between-group estimates. Mean eGFR decline appeared to be non-progressive to a period of 36 months, as assessed with the additional follow-up of the two active PrEP arms beyond July 2011. The majority of creatinine elevations observed were self-limited and were not confirmed on subsequent measurement, and the occurrence of clinically relevant decline in eGFR (i.e. ≥25% eGFR decline from baseline) was low. In the two subjects who developed eGFR <60 min/mL/1.73m^2, eGFR rebounded to >60 min/mL/1.73m^2 within four weeks after drug discontinuation. There was no evidence of substantial increase in clinically relevant eGFR decline related to PrEP compared to placebo although, given the 95% confidence intervals, an increase in absolute rate of a ≥25% eGFR decline as high as 1% per year that could be attributed to PrEP cannot be ruled out.

Drug exposure is an important determinant of both PrEP efficacy and assessment for safety. Adherence in the Partners PrEP Study was the highest of any published PrEP clinical trial:[1,55] tenofovir was detectable in plasma in 82% of a randomly selected cohort

of subjects and 17% of those samples with no detected drug were a result of protocol-defined drug holds.[56] Our findings, which enriched for drug exposure in the primary analysis by limiting to per-protocol periods, are thus encouraging in demonstrating that clinically relevant eGFR decline was rare in the context of high exposure to PrEP.

Our study provides both new and complimentary evidence to the recent analysis from the iPrEx study,[57] a PrEP trial among men who have sex with men, in which FTC-TDF PrEP was associated with a small but statistically significant decrease in calculated creatinine clearance. However, an important limitation of that analysis was that PrEP adherence, based on detection of tenofovir in plasma, was estimated to be only ~50% in iPrEx.

The results should be interpreted in light of the following limitations. First, creatinine-based GFR estimating equations are less accurate in persons with low creatinine generation, including those with low muscle mass, muscle wasting, or reduced meat intake, which may be more common in African individuals. The CKD-EPI equation has demonstrated high accuracy in African populations, and intra-individual changes in eGFR are less susceptible to this limitation of creatinine-based estimates. Second, long-term treatment effects beyond the study period cannot be ascertained. However, it is reassuring that in a large observational study with long-term follow-up (median: 7.9 years) of HIV infected individuals on TDF-containing combination antiretroviral therapy, most of the observed eGFR loss occurred during the first year of TDF exposure and stabilized after 2 years[27]. In our study, mean eGFR decline appeared to stabilize after

the first year of observation and then waned over time. Third, post-randomization censoring has the potential to introduce selection bias and/or confounding. However, the consistency of the primary analysis estimates with marginal structural models estimates lends confidence to our findings. Fourth, against a low background level of a ≥25% eGFR decline (i.e. 0.83% per year recorded in the placebo group) we had only the ability to detect large increases in the risk of ≥25% eGFR decline. However, the low absolute rates of ≥25% eGFR decline recorded in the active arms with additional follow-up (median of 36 months in the active PrEP arms) is encouraging. Fifth, the study required persons with normal renal function at entry, and PrEP effects among subpopulations with co-morbidities or concurrent nephrotoxic medications could not be fully evaluated. Lastly, the current study did not evaluate changes in proximal tubular function, another potential consequence of TDF exposure. A recent sub-study in iPrEx did not show evidence of nephrotubulopathy,[57] and we observed no significant difference in graded abnormalities in serum phosphorus between the PrEP and placebo.[1] Whether TDF-based PrEP causes early proximal tubular injury in HIV uninfected individuals warrants additional evaluation.

In conclusion, in this large randomized placebo-controlled trial among uninfected African men and women, with median follow-up of 18 months and maximum follow-up of 36 months, daily oral TDF-based PrEP was associated with a small but non-progressive decline in eGFR that was not accompanied by a substantial increase in the risk of clinically relevant eGFR decline. Our data support the safety of TDF-based PrEP in heterosexual populations as part of a comprehensive HIV-1 prevention package.

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*Study concept and design**:*** Mugwanya, Baeten, Celum, Donnell, Wyatt.

Acquisition, analysis, or interpretation of data: Mugwanya, Baeten, Celum, Donnell, Wyatt, Mugo, Kiarie, Tappero, Ronald.

Drafting of the manuscript: Mugwanya, Baeten.

Critical revision of the manuscript for important intellectual content: Mugwanya, Baeten, Celum, Donnell, Wyatt, Mugo, Kiarie, Tappero, Ronald.

Statistical analysis: Mugwanya, Baeten, Donnell, Wyatt.

Administrative, technical, or material support: Mugwanya, Baeten, Celum, Donnell, Wyatt, Mugo, Kiarie, Tappero, Ronald.

Study supervision: Mugwanya, Baeten, Celum, Donnell, Mugo, Kiarie, Tappero, Ronald.

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Partners PrEP Study Team:

University of Washington Coordinating Center and Central Laboratories, Seattle, USA: Connie Celum (principal investigator, protocol co-chair), Jared M. Baeten (medical director, protocol co-chair), Deborah Donnell (protocol statistician), Robert W. Coombs, Lisa Frenkel, Craig W. Hendrix, Jairam R. Lingappa, M. Juliana McElrath.

Study sites and site principal investigators: Eldoret, Kenya (Moi University, Indiana University): Kenneth H. Fife, Edwin Were; Kabwohe, Uganda (Kabwohe Clinical Research Center): Elioda Tumwesigye; Jinja, Uganda (Makerere University, University of Washington): Patrick Ndase, Elly Katabira; Kampala, Uganda (Makerere University): Elly Katabira, Allan Ronald; Kisumu, Kenya (Kenya Medical Research Institute, University of California San Francisco): Elizabeth Bukusi, Craig R. Cohen; Mbale, Uganda (The AIDS Support Organization, CDC-Uganda): Jonathan Wangisi, James D. Campbell, Jordan W. Tappero; Nairobi, Kenya (University of Nairobi, University of Washington): James Kiarie, Carey Farquhar, Grace John-Stewart; Thika, Kenya (University of Nairobi, University of Washington): Nelly R. Mugo; Tororo, Uganda (CDC-

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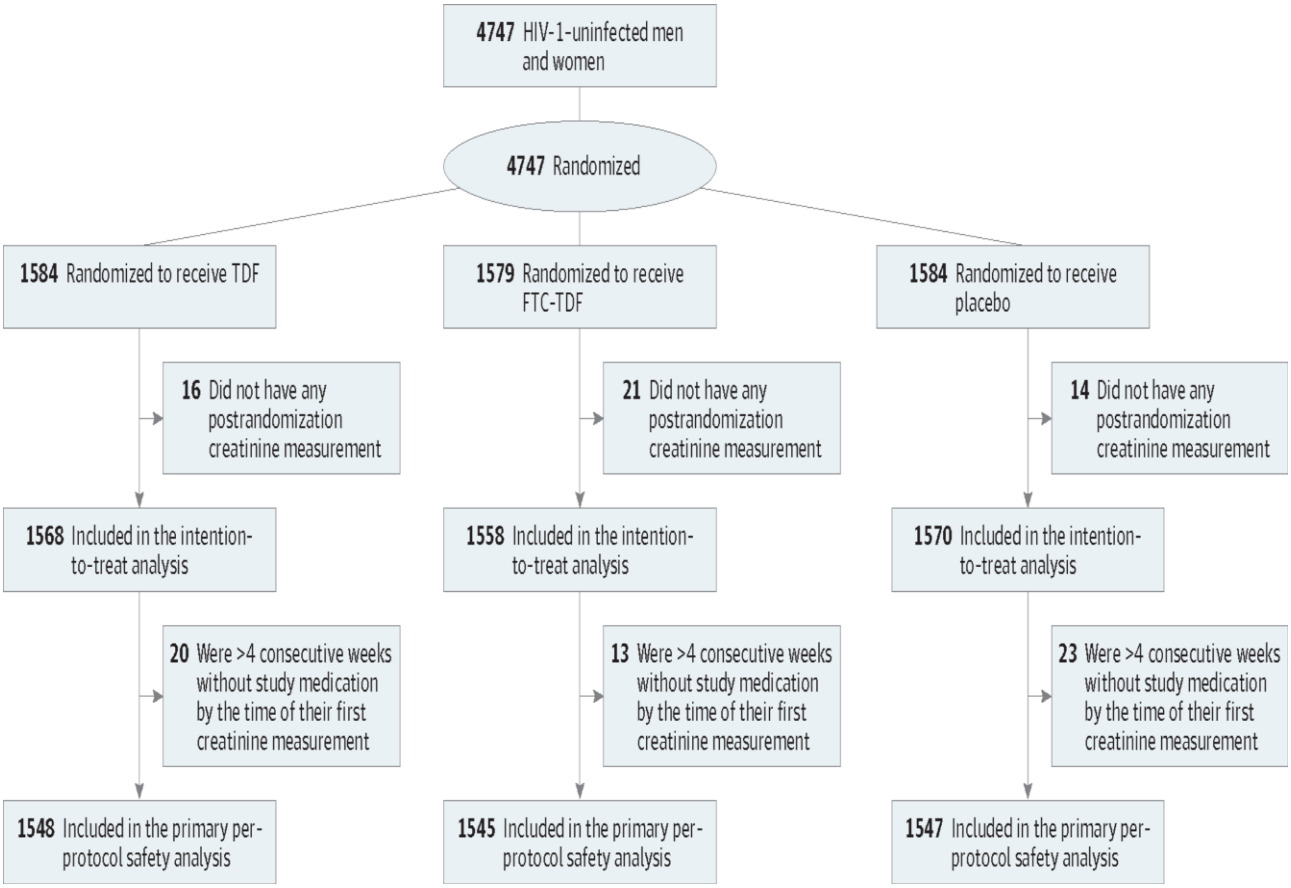


Figure 0:1. Sequence of randomization and subsequent exclusion or study completion of study participants

FTC indicates emtricitabine; TDF, tenofovir disoproxil fumarate.

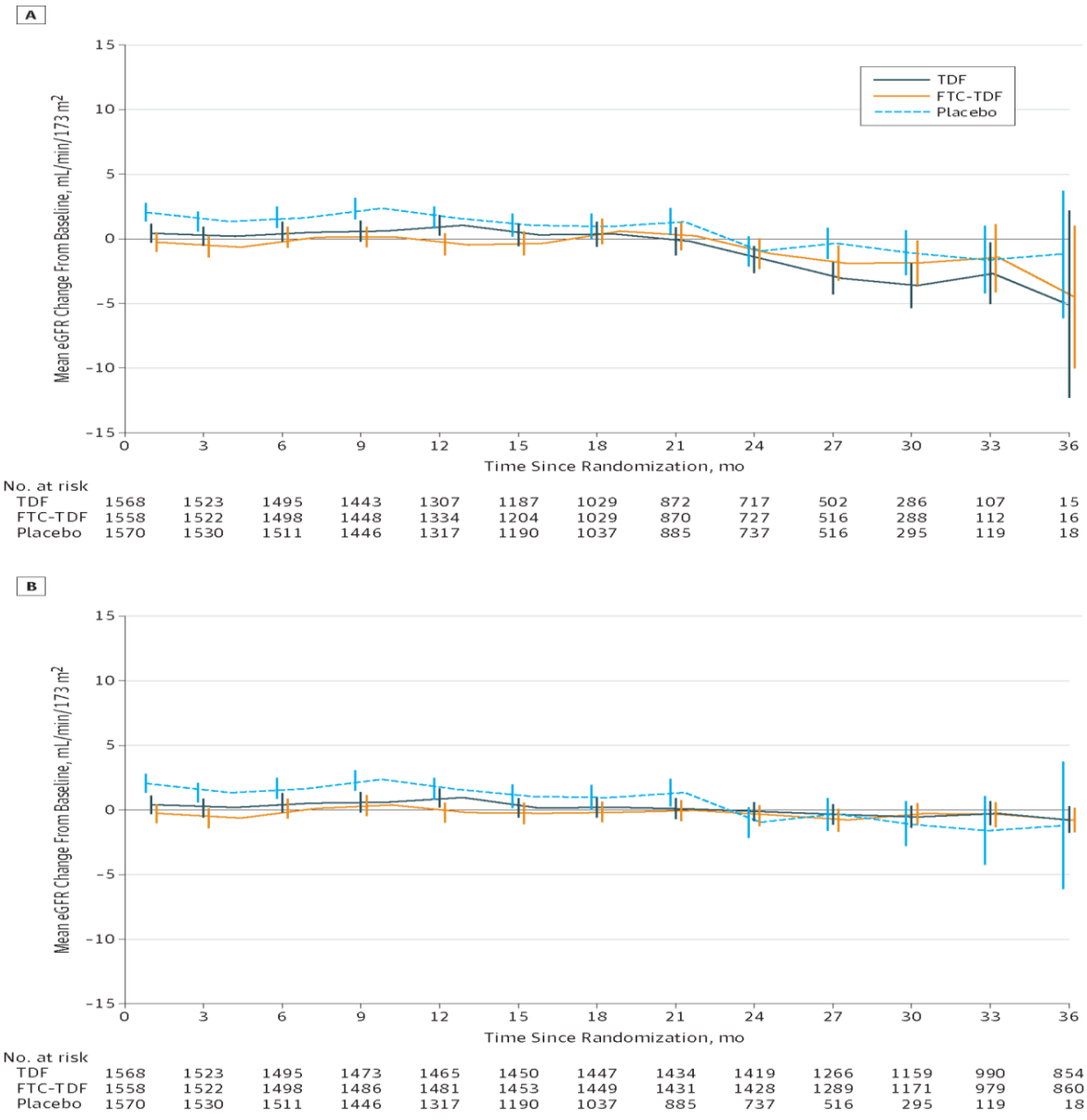
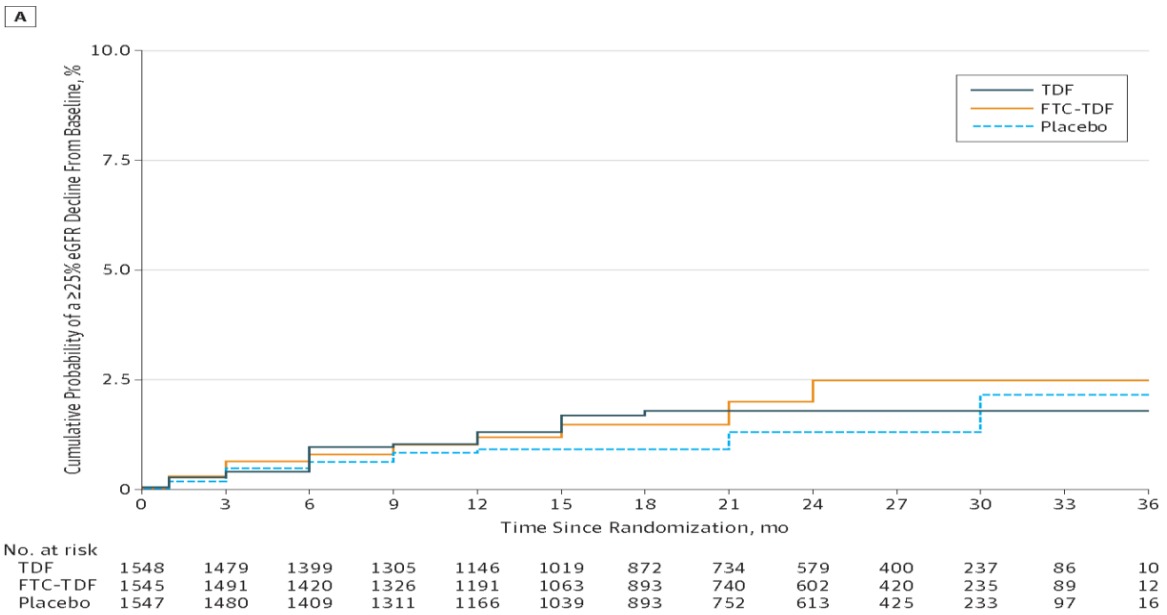


Figure 0:2. Variation over time in crude mean estimated glomerular filtration rate changes from baseline according to treatment group.
A, Graph represents all data collected through July 2011, when the trial's placebo arm was discontinued; because of truncation of follow-up time in July 2011, few participants had achieved more than 30 months of follow-up. B, Graph represents crude mean eGFR changes from baseline that includes additional follow-up of the TDF and FTC-TDF arms beyond July 2011. The placebo group contributed person-time up to only July 2011. A and B, Vertical lines indicate 95% CIs; eGFR, estimated glomerular filtration rate; FTC, emtricitabine; TDF, tenofovir disoproxil fumarate.



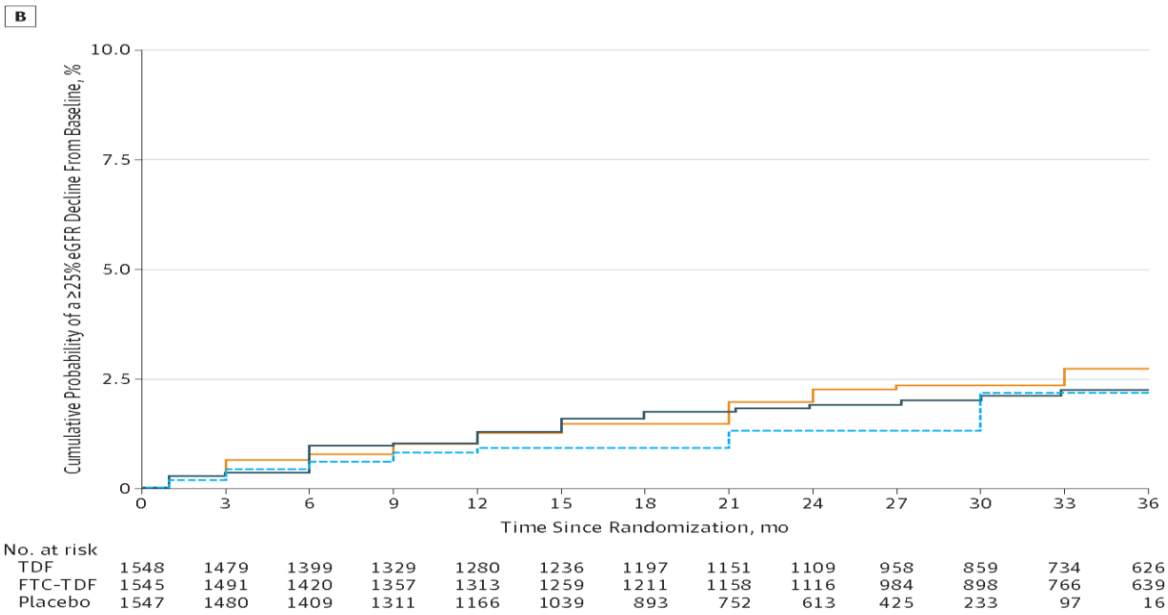


Figure 0:3. Cumulative probability of ≥25% estimated glomerular filtration rate changes from baseline according to treatment group.

A, Estimates for the primary per-protocol safety analysis including data accrued up to July 2011, when the placebo arm was discontinued. B, Estimates for the sensitivity analysis that included additional follow-up of the TDF and FTC-TDF arms beyond July 2011, with the placebo arm data truncated at July 10, 2011. A and B, Failure function was calculated over full data and evaluated at indicated times; it is not calculated from aggregates of number of persons shown on the x-axis plots. eGFR indicates estimated glomerular filtration rate; FTC, emtricitabine; TDF, tenofovir disoproxil fumarate.

Table 0:1. Enrollment characteristics according to treatment group

Characteristic	FTC-TDF (n=1545)	TDF (n=1548)	Placebo (n=1547)
Age (years)			
Mean (range)	35 (18-64)	34 (18-64)	35 (18-64)
≤24	11%	12%	11%
25-34	44%	45%	43%
35-44	32%	30%	33%
≥45	13%	13%	13%
Male	64%	62%	61%
Creatinine (mg/dL)	0.78 ± 0.15	0.78 ± 0.15	0.78 ± 0.15
eGFR (mL/minute/1.73m^2)			
Mean	129 ± 17	130 ± 17	129 ± 17
eGFR ≥90	98%	97%	98%
Weight (kg)			
Mean	61 ± 10	61 ± 10	61± 11
>50kg	87%	86%	87%
Systolic blood pressure ≥140mmHg	5%	5%	6%
Diastolic blood pressure ≥90mmHg	3%	3%	5%

Unless stated, statistics are mean ±standard deviations for continuous covariates and percentages for categorical covariates. FTC denotes emtricitabine TDF denotes tenofovir disoproxil fumarate.

Table 0:2. Estimated mean eGFR difference from baseline, according to treatment group

	Number of individuals evaluated; within group estimated mean eGFR change from baseline, (mL/min/1.73 m²)[d]						***Attributable mean eGFR difference* mL/min/1.73 m² *(95%CI)[d]***	
Characteristic	***FTC-TDF***		***TDF***		***Placebo***		***TDF vs. placebo***	***FTC-TDF vs. placebo***
A. Primary analysis- overall treatment effect								
Overall per-protocol	1545;	-0.22	1548;	+0.14	1547;	+1.37	-1.23 (-2.06, -0.40); p<0.01	-1.59 (-2.44, -0.74); p<0.01
B. Sensitivity analyses for overall treatment effect								
MSM[a] per-protocol	1545	-0.21	1548	+0.15	1547	+1.38	-1.23 (-2.06, -0.40); p<0.01	-1.59 (-2.44, -0.74); p<0.01
Intention-to-treat[b]	1558;	-0.17	1568;	+0.18	1570;	+1.41	-1.23 (-2.06, -0.41); p<0.01	-1.59 (-2.44, -0.74); P<0.01
ITT extended follow-up[c]	1558;	-0.08	1568;	+0.32	1570;	+1.28	-0.96 (-1.78, -0.14); p=0.02	-1.36 (-2.20, - 0.52); p<0.01
C. Treatment effect among subgroups (per-protocol approach)								
Sex								
Male	988;	+0.25	962;	+0.66	936;	+1.75	-1.09 (-2.09, -0.08); p=0.03	-1.50 (-2.53, -0.49); p<0.01
Female	557;	-0.69	586;	-0.43	611;	+1.04	-1.47 (-2.92, -0.02); p=0.05	-1.73 (-3.23, -0.23); p=0.02
Age								
18-34yrs	846;	-0.39	879;	+0.29	834;	+1.28	-0.99 (-2.19, 0.21); p=0.10	-1.67 (-2.88, -0.46); p<0.01
35-44yrs	491;	-0.21	471;	+0.33	508;	+1.78	-1.45 (-2.87, -0.02); p=0.05	-1.99 (-3.45, -0.54); p<0.01
≥45yrs	208;	+0.27	198;	-0.82	205;	+0.76	-1.58 (-3.49, 0.34); p=0.11	-0.49 (-2.56, 1.58); p=0.64
D: Treatment effect by month since randomization (per-protocol approach)								

1 month	1545;	-0.19	1548;	+0.53	1547;	+2.23	-1.70 (-2.68, -0.72); p<0.01	-2.42 (-3.42, -1.43); p<0.01
3 months	1495;	-0.58	1481;	+0.21	1476;	+1.43	-1.22 (-2.25, -0.19); p=0.02	-2.01 (-3.08, -0.95); p<0.01
6 months	1428;	+0.09	1402;	+0.44	1410;	+1.51	-1.07 (-2.14, <-0.01); p=0.05	-1.42 (-2.51, -0.33); p=0.01
12 months	1203	-0.41	1159	+1.03	1173	+1.60	-0.57 (-0.72, 0.58); p=0.33	-2.01 (-3.20, -0.83); p<0.01
15 months	1078;	-0.08	1031;	+0.42	1046;	+0.90	-0.48 (-1.71, 0.74); p=0.44	-0.98 (-2.25, 0.28); p=0.13
18 months	905;	+0.90	888;	+0.66	899;	+1.16	-0.50 (-1.89, 0.88); p=0.47	-0.26 (-1.65, 1.13); p=0.71
24 months	614;	-1.01	589;	-1.40	621;	-0.59	-0.81 (-2.39, 0.77); p=0.31	-0.42 (-2.11, 1.27); p=0.63

The primary approach was a per-protocol analysis censoring any visits occurring after >4 consecutive weeks off study medication.

[a] MSM: Marginal structural models. The marginal structural models used time-dependent stabilized weights (mean 1.00, range 0.99-1.03), which were estimated using pooled logistic regression models with censoring indicator as the outcome and prior visit histories of the time-varying covariates, creatinine clearance and serum creatinine, as 3 knot restricted cubic splines at 5th, 50th, and 95th percentiles plus treatment arm, sex, site, age, baseline eGFR, and time since randomization as part of the numerator model.

[b] Intention-to-treat analysis includes all randomized participants with at least one post-randomization serum creatinine measurement regardless of time off study medication, for data collected through July 2011.

[c] ITT extended follow-up analysis is an intention-to-treat approach that includes the additional follow-up time of persons randomized to TDF and FTC-TDF groups beyond July 2011 when the placebo arm was discontinued. During the extended follow-up, both investigators and participants remained blinded to the type of PrEP drug the participant was receiving. Placebo groups contributed records only up to July 2011.

[d] Attributable mean eGFR difference represents the difference in mean eGFR change from baseline between the respective active PrEP treatment group and the placebo group. All subgroups and the treatment by month estimates are for the per-protocol approach. Estimates were generated from linear regression fit using generalized estimating equations. Models were adjusted for sex, indicator for site to account for heterogeneity in serum creatinine assaying, continuous age and time since randomization as 3 knot restricted cubic splines at 5th, 50th, and 95th percentiles. Baseline body mass index, elevated blood pressure or non-steroidal inflammatory drug use did not modify PrEP effects (p>0.05 for all) and their addition as covariates did have substantial effect on the estimates. Treatment effects by visitmonth since randomization were generated from

treatment by categorical time interaction. P-values are two-sided testing the null hypothesis of no treatment effects. FTC denotes emtricitabine TDF denotes tenofovir disoproxil fumarate

Table 0:3. Absolute incidence rates and hazard ratios for a ≥25% eGFR decline from baseline overall and among subgroups, according to treatment study group.

	No. of events/total person-years			***Absolute incidence rate difference per 100 person-years[a] (95%CI)***		***Adjusted hazard ratio[e] (95%CI); p-value***	
Approach	***FTC-TDF***	***TDF***	***Placebo***	***FTC-TDF vs. Placebo***	***TDF vs. Placebo***	***TDF vs. Placebo***	***FTC-TDF vs. Placebo***
A. Primary per-protocol analysis -overall treatment effect							
Per-protocol	27/2184.1	23/2133.3	18/2174.3	0.41 (-0.19, 1.01)	0.25 (-0.33, 0.83)	1.33 (0.70, 2.48); p=0.37	1.45 (0.79, 2.64); p=0.23
B. Sensitivity analyses							
MSM per-protocol	27/2184.1	23/2133.3	18/2174.3	0.41 (-0.19, 1.01)	0.25 (-0.33, 0.83)	1.33 (0.71, 2.51); p=0.38	1.45 (0.80, 2.63); p=0.23
Repeated events[b]	37/2206.9	31/2154.3	26/2188.1	0.49 (-0.22, 1.20)	0.25 (-0.43, 0.93)	1.22 (0.55, 2.67); p=0.63	1.38 (0.68, 2.79); p=0.37
Intention-to-treat[c]	28/2445.2	25/2431.6	19/2460.7	0.37 (-0.18, 0.92)	0.26 (-0.28, 0.79)	1.37 (0.75, 2.50); p=0.31	1.44 (0.80, 2.59); p=0.22
ITT extended follow-up[d]	37/3731.2	32/3733.0	19/2460.7	0.22 (-0.25, 0.69)	0.09 (-0.37, 0.54)	1.38 (0.78, 2.46); p=0.27	1.54 (0.88, 2.70); p=0.13
C. Treatment effect among subgroups (per-protocol approach)							
Sex							
Male	12/1392.1	8/1349.2	8/1362.4	0.27 (-0.36, 0.91)	0.01 (-0.57, 0.58)	1.04 (0.39, 2.78); p=0.94	1.41 (0.5, 3.45); p=0.46
Female	15/792.0	15/784.0	10/811.9	0.66 (-0.56, 1.89)	0.68 (-0.55, 1.91)	1.51 (0.68, 3.38); p=0.31	1.56 (0.70, 3.48); p=0.28
Age groups							
18-34yrs	9/1065.1	15/1089.5	7/1056.9	0.18 (-0.56, 0.92)	0.35 (-0.43, 1.12)	1.54 (0.60, 3.98); p=0.37	1.37 (0.5, 3.67); p=0.54

35-44yrs	13/747.1	9/714.7	9/755.1	0.55 (-0.68, 1.77)	0.07 (-1.07, 1.20)	1.07 (0.42, 2.69); p=0.89	1.56 (0.67, 3.67); p=0.30
≥45yrs	5/371.9	3/329.1	2/362.3	0.79 (-0.61, 2.20)	0.35 (-0.92, 1.64)	1.46 (0.24, 8.76); p=0.68	2.11 (0.4,10.94); p=0.37

The primary approach was a per-protocol analysis censoring visits occurring after >4 consecutive weeks off study medication.

MSM: Marginal structural models weighted by inverse probability censoring weights (mean weight 1, range 0.99, 1.02); details of weight estimation provided in etable 1b.

[a] Absolute incidence rate difference represents the difference of incidence rate in the placebo arm from the incidence rate of the respective active PrEP arm. The rates and rate differences reported for the marginal structural models results are unweighted as in the primary approach.

[b] Analysis of repeated events of ≥25% eGFR decline in a per-protocol approach using Andersen Gill counting process approach in Cox- regression model. Given that changes in eGFR is a continuum of cumulative biological process for which the true start or end of at-risk periods for each repeated event is nearly impossible to establish, the reported rates in the repeated events approach should be interpreted rates of episodes of ≥25% eGFR decline but not as true incidence rates.

[c] Intention-to-treat analysis included all randomized participants with at least one post-randomization serum creatinine measurement regardless of the time off study medication, for data collected through July 2011.

[d] ITT extended follow-up is an intention-to-treat approach that includes additional follow-up time of persons randomized to TDF and FTC-TDF groups beyond July 2011 when the placebo arm was discontinued by the DSMB. The proportion of patients who developed a ≥25% eGFR decline from baseline was consistent with that recorded in the primary per-protocol analysis (i.e 1.3% for TDF, 1.3%, and 0.9% for the placebo, by 12 months; 1.9% for TDF, 2.3%, and 1.3% for the placebo by 24 months; and 2.2% for TDF, 2.8%, and 2.2% by 36 months). During the additional follow-up both the investigators and participants remained blinded to the type of PrEP drug the participant was receiving. Placebo groups contributed records only up to July 2011.

[e] Hazard ratios estimated using Cox proportional hazards models stratified according sex, age groups, and site to account for laboratory heterogeneity in serum creatinine estimation. For subgroups, the group evaluated was dropped from the stratification. All subgroups estimates are for the per-protocol approach. Models were adjusted for baseline eGFR as 3 knot restricted cubic splines at 5^{th}, 50^{th}, and 95^{th} percentiles. All p-values are two-sided testing the null hypothesis of no treatment effects. FTC denotes emtricitabine and TDF denotes tenofovir disoproxil

Effect of emtricitabine-tenofovir disoproxil fumarate pre-exposure prophylaxis on the risk of proximal tubular dysfunction associated in HIV-uninfected men and women

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Effect of Emtricitabine-Tenofovir Disoproxil Fumarate Pre-Exposure Prophylaxis on the Risk of Proximal Tubular Dysfunction in HIV-uninfected Men and Women

Kenneth Mugwanya[1, 2, 3], MBChB, MS; Jared Baeten[1, 2,4], MD, PhD; Connie Celum[1, 2,4], MD, MPH; Deborah Donnell[5], PhD; Thomas Nickolas[6], MD, MS; Nelly Mugo[2, 7], MBChB, MPH; Andrea Branch[8], PhD**;** Jordan Tappero[9], MD, MPH; James Kiarie[10], MBChB, MPH; Allan Ronald[11], MD; Michael Yin[12], MD, MS; Christina Wyatt[13], MD, MSc, for the Partners PrEP Study Team*

Affiliations:

From the Departments of [1]Epidemiology, [2]Global Health, and [4]Medicine University of Washington, Seattle, USA; [3]Division of Disease Control, School of Public Health, Makerere University, Kampala, Uganda; [5]Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, USA; [6]Department of Medicine, Divisions of [6]Nephrology and [12]Infectious Diseases, Columbia University Medical Center, New York, USA; [7] Kenya Medical Research Institute, Nairobi, Kenya; Department of Medicine, Divisions of [8]Liver Diseases and [13]Nephrology Icahn School of Medicine at Mount Sinai, New York; [9] Division of Global Health Protection, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, USA; [10]Department of Obstetrics and Gynecology, University of Nairobi, Nairobi, Kenya; [11]Department of Medicine, University of Manitoba, Winnipeg, Canada; *Members of the Partners PrEP Study Team listed in the footnote page.

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Correspondence to: Kenneth K. Mugwanya, University of Washington 325 Ninth Avenue UW Box 359927 Seattle, WA, 98104 Phone: (206) 520-3806Fax: (206) 520-3831 Email: mugwanya@uw.edu

Abstract

Objective: Tenofovir disoproxil fumarate (TDF) is associated with proximal tubular dysfunction (tubulopathy) when used in HIV treatment. We evaluated whether TDF causes tubulopathy when used as HIV pre-exposure prophylaxis (PrEP) and whether tubulopathy predicts clinically relevant decline (≥25%) in estimated glomerular filtration rate (eGFR).

Methods: A subgroup analysis of the Partners PrEP Study, a randomized, placebo-controlled trial of daily oral TDF and emtricitabine (FTC)-TDF among HIV-uninfected African men and women (Clinicaltrials.gov number NCT00557245). Tubulopathy was assessed in concurrently obtained urine and serum at the 24-month or last on-treatment visit, pre-defined as ≥2 of: tubular proteinuria, euglycemic glycosuria, increased urinary phosphate or uric acid excretion.

Results: Of 1549 persons studied (776 on FTC-TDF, 773 on placebo), 64% were male and median age was 37-years. Over median 24-months of study-drug exposure, the frequency of tubulopathy was 1.7% in FTC-TDF versus 1.3% for placebo [odds ratio (OR), 95%CI: 1.30 (0.52, 3.33); p=0.68]. Tubulopathy occurred in 2 of 52 (3.8%) persons with ≥25% eGFR decline versus 3 of 208 (1.4%) without ≥25% eGFR decline [adjusted OR 95%CL: 1.39 (0.10, 14.0); p >0.99].

Conclusions: Daily oral FTC-TDF PrEP was not significantly associated with tubulopathy over 24 months nor did tubulopathy predict clinically relevant eGFR decline.

Key words: PrEP; TDF toxicity, proximal tubular dysfunction, TDF nephrotoxicity

Introduction

Pre-exposure prophylaxis (PrEP) with tenofovir disoproxil fumarate (TDF), alone or in combination with emtricitabine (FTC-TDF), protects against HIV acquisition in diverse at-risk populations [1-4]. Although TDF is well tolerated, proximal tubular dysfunction has been observed more frequently in HIV-infected persons using TDF-containing antiretroviral regimens compared to persons using non-TDF-containing regimens[26,27,29,58-60]. Clinically significant proximal tubular dysfunction, or Fanconi syndrome, is a rare but serious complication characterized by low molecular-weight proteinuria, euglycemic glycosuria, hypophosphatemia, phosphaturia, metabolic acidosis, and hypouricemia. In clinical trials [1-3,15,16,61], TDF-based PrEP was not associated with overt kidney toxicity as assessed by serum creatinine, but early proximal tubular injury can occur without severe decline in the glomerular filtration rate (GFR) [58]. No study has assessed the effect of TDF-based PrEP on proximal tubular function in HIV-uninfected women. An optional sub-study of the iPrEx Study previously demonstrated a low rate of proximal tubulopathy in HIV-uninfected men assigned to TDF-based PrEP; however, a substantial number of participants randomized to FTC-TDF were not adherent to PrEP as measured by plasma tenofovir concentrations in the primary study [57]. In a randomized placebo-controlled comparison, we investigated whether daily FTC-TDF PrEP causes proximal tubular dysfunction among HIV-uninfected men and women with high adherence to PrEP, and whether tubular dysfunction predicts a subsequent clinically relevant decline in estimated GFR (eGFR).

Methodology

Study population

This is a subgroup analysis of the Partners PrEP Study, a large randomized, placebo-controlled trial of daily oral TDF and FTC-TDF PrEP (Clinicaltrials.gov number NCT00557245)[1]. The study enrolled 4747 heterosexual HIV serodiscordant couples at nine sites in Kenya and Uganda between July 2008 and November 2010. Eligible HIV-uninfected participants were ≥18 years of age, did not have active hepatitis B infection, and had Cockcroft-Gault creatinine clearance (CrCl) ≥60 mL/min). HIV-uninfected partners were randomly assigned in a 1:1:1 ratio to one of three study groups: TDF 300 mg daily, FTC-TDF 300-200 mg daily, or matching placebo, and were followed monthly up to 36 months. Serum and urine samples were collected and archived at baseline, month 3, and then annually to assess proximal tubular function. In July 2011, the independent data and safety monitoring board (DSMB) recommended that the placebo arm be discontinued owing to definitive demonstration of PrEP efficacy. The study protocol was approved by the University of Washington Human Subjects Review Committee and ethics review committees at collaborating institutions. All participants provided written informed consent.

Approach for the current analysis

We used two complementary analyses to answer two related questions: 1) a subgroup cohort analysis of HIV-uninfected men and women randomly assigned to FTC-TDF versus placebo to determine whether FTC-TDF PrEP causes proximal tubular dysfunction, as the primary aim, and 2) a nested case-control analysis of persons on

TDF or FTC-TDF to investigate whether proximal tubular dysfunction predicts subsequent clinically relevant eGFR decline (≥25%), as the secondary aim.

Intervention and adherence assessment

For the cohort analysis, the intervention was a comparison of the frequency of proximal tubular dysfunction between persons who were randomized to FTC-TDF versus placebo. Our goal was to maintain randomized group assignment in the selected cohort while maximizing the duration of drug exposure in the selected cohort to reflect the cumulative nature of TDF toxicity. The primary selection criterion for inclusion in the cohort analysis was the chance to have at least 24 months of study follow-up by July 10, 2011 when the study placebo arm was suspended by the DSMB. This selection criterion is based on a baseline variable "date of enrollment into the study" and thus preserves the randomized group assignment in the selected sub-group. FTC-TDF was chosen as it is the US FDA-approved drug for PrEP, and we assumed that potential safety signals would be equally or potentially more prevalent in persons on dual co-formulation than on single agent. The nested case-control analysis included persons on the TDF or FTC-TDF arms to capture all cases of severe eGFR decline (≥25%) with TDF exposure observed in the Partners PrEP study cohort. Adherence to study medication was assessed by pill counts of returned bottles at each monthly visit, and blood samples for tenofovir level were collected in a random sample of participants at months 1, 3, and bi-annually. Plasma tenofovir concentrations were previously determined in archived plasma samples by ultra-performance liquid chromatography-mass spectrometry assay methods, with detectable plasma tenofovir concentration defined as >0.31 ng/mL, consistent with other PrEP trials [3,16].

Participant selection

Prospective cohort analysis: Eligible persons were HIV-uninfected men and women who were randomized to FTC-TDF and placebo arms in the Partners PrEP Study at least 24 months prior to July 10, 2011, when the placebo arm was discontinued by the DSMB. Our goal was to maintain the randomized group assignment while enriching for a long duration of drug exposure to reflect the cumulative nature of TDF-related toxicity. All eligible persons with concurrently collected urine and serum samples were included. We selected archived serum and urine samples from a single on-treatment visit, either the 24-month visit or, for those who did not achieve the Month 24 visit, the last on-treatment visit. Samples collected after HIV seroconversion were not included.

Nested case-control analysis (Figure 1): Clinically relevant eGFR decline was defined as a confirmed ≥25% decline from baseline, an established criterion for the diagnosis of acute kidney injury and a marker of increased morbidity and mortality[37-40]. As previously reported [62], 69 persons in the two active PrEP arms experienced ≥25% eGFR decline from baseline, confirmed by repeat testing ("cases"): 37 in the FTC-TDF and 32 in the TDF arm. All cases in the active PrEP arms with concurrent urine and serum samples at any visit preceding the case diagnosis visit were selected; for cases with samples available at more than one visit, the most proximal visit sample was selected. Controls were participants randomized to TDF and FTC-TDF who never experienced ≥25% eGFR decline and who had concurrently collected urine and serum samples. For each selected case, 4 controls were randomly selected and were frequency-matched to cases by assigned treatment group (TDF or FTC-TDF) and duration of drug exposure.

Measurement and definitions of proximal tubular dysfunction

Serum assays for tubular function included creatinine, phosphorus, glucose, and uric acid; and urine assays included creatinine, phosphorus, glucose, uric acid, albumin, and total protein. Proximal tubulopathy was pre-defined as the occurrence of ≥2 of the following markers of proximal tubular dysfunction at the same time point: tubular proteinuria, defined as proteinuria of >200mg/g with urine albumin: total protein ratio <0.4; euglycemic glycosuria, defined as positive urine glucose (≥10 mg/dL) with normal random serum glucose (<126 mg/dl); increased urinary excretion of phosphorus, defined as a fractional tubular resorption of phosphorus (%TRP) <82%; and increased urinary excretion of uric acid, defined as fractional excretion of uric acid >15%. To characterize low grade phosphate and glucose wasting, two cardinal features of Fanconi syndrome, we also computed the maximum rate of tubular phosphate reabsorption to the glomerular filtration rate (TmP/GFR) using the algorithm derived by Kenny and Glen[63-65], with values of <2.6 mg/dL (0.80mmol/L) considered abnormal, and fractional resorption of glucose (%TRG), with values <100% considered glucose wasting. In the nested case-control analysis, persons who experienced ≥25% eGFR decline were identified from our previous work on eGFR changes associated with TDF-based PrEP [62]. eGFR was calculated from serum creatinine using the Chronic Kidney Disease Epidemiology Collaboration equation [34]. Testing for tubular parameters was performed by the Laboratory Medicine Research Testing Services of the University of Washington.

Statistical analysis:

For the cohort analysis, the primary outcome was proximal tubulopathy, predefined as the composite of ≥2 markers of proximal tubular dysfunction and compared between those assigned FTC-TDF PrEP versus placebo. We tested for the difference in proportions using exact or asymptotic methods where appropriate. Additional analysis compared the frequency of the individual markers of proximal tubular dysfunction between FTC-TDF versus placebo. In sensitivity analyses, we used more stringent definitions of abnormal values for phosphate resorption (%TRP <95% with serum phosphate <2.6 mg/dL or TmP/GFR <0.80 mmol/L) and glucose resorption (%TRG <100%).

For the nested case-control analysis, the primary exposure of interest was proximal tubulopathy as defined in the cohort analysis, and cases (who experienced ≥25% eGFR decline) were compared to controls using logistic regression. In addition to the primary composite variable defining proximal tubulopathy, we evaluated whether any of the individual markers of proximal tubular dysfunction separately predict a ≥25% eGFR decline. Each model was adjusted for sex, age, body mass index, and indicator for elevated systolic blood pressure. Analyses were conducted using SAS software, version 9.4 (SAS Institute Inc).

Results

Of the 3163 HIV-uninfected participants randomized to the FTC-TDF and placebo groups in the Partners PrEP Study, 1576 were randomized at least 24 months prior to

termination of the placebo arm and thus met the primary selection criterion; 1549 of those (98%) had concurrently obtained urine and serum and were included in the cohort analysis: 776 in the FTC-TDF and 773 in the placebo arm (**Error! Reference source not found.**). Of these 1549, 64% were male, median age was 37 years (range, 18-64), and baseline characteristics were comparable in the two treatment groups (Table 0:1). Overall, 1394 (90%) and 93 (6%) persons had tubular testing performed on their month 24 and 12 sample, respectively, with similar proportions across treatment groups.

In the nested case control approach, 52 cases of ≥25% eGFR decline from baseline in the active PrEP arms were frequency matched to 208 controls (i.e., participants in the active PrEP arms who never experienced a ≥ 25% eGFR decline from baseline) in a 1:4 ratio; 17 cases were excluded due to absence of concurrent urine and serum samples. Compared to controls, cases tended to be females, of older age, and with lower baseline CrCl, but were comparable on all other baseline characteristics (Table 0:2). Median follow-up at case diagnosis was 15 months. Overall, excluded cases (n=17) were comparable to included cases on baseline characteristics (data not shown).

Effect of FTC-TDF PrEP on proximal tubular function

Proximal tubulopathy, defined as a composite of ≥2 markers of proximal tubular dysfunction as described in the Methods, was rare in this study population (Table 0:3): 13 (1.7%) participants in the FTC-TDF group compared to 10 (1.3%) participants in the placebo group [odds ratio (95% confidence interval): 1.30 (0.52, 3.33); p=0.68]. Multivariate analysis with sex and baseline age, CrCl, indicator for elevated systolic blood pressure, and body mass index did not have substantial effect on the estimates

(data not shown). Sensitivity analyses with conservative definitions for phosphate and glucose wasting yielded similar results, as did analysis excluding 68 participants with >4 consecutive weeks off study medication for any reason during the 24-month period. The distributions of individual biomarkers of phosphate and glucose resorption were similar in the two treatment groups; however, tubular proteinuria [7.3% (57) vs 4.0% (31); p=0.01] and increased urinary excretion of uric acid [3.5% (27) vs 1.3% (10); p=0.001] occurred more frequently in the FTC-TDF group versus the placebo group. Overall, drug exposure was high in this cohort; tenofovir was detectable in 787 of 1028 (77%) plasma samples available from a subset of 303 participants in the FTC-TDF group [median tenofovir plasma concentration: 67 ng/mL (IQR, 1.89-101.0); >48 ng/mL is indicative of daily dosing].

Association of proximal tubular dysfunction with clinically relevant decline in eGFR

In the nested case-control analysis, proximal tubulopathy occurred in 2 of 52 (3.8%) cases of ≥25% eGFR decline compared to 3 of 208 (1.4%) controls [adjusted odds ratio, 95% confidence interval aOR, (95% CI): 1.39 (0.10, 14.1); p >0.99] (Table 0:4). Sensitivity analyses with more conservative definitions of proximal tubulopathy composite outcome yielded similar findings. Conversely, individual markers of proximal tubular dysfunction as defined in the methods Section appeared to be associated with elevated odds for ≥25% eGFR decline but no significant statistical differences were recorded (p>0.05 for all assessed markers; Table 0:4). Overall, baseline covariates significantly associated with increased risk of ≥25% eGFR decline were female sex

[aOR, (95% CI): 3.03 (1.48, 6.21); p=0.002] and older age [aOR, (95%CI) for every 5-year age increase: 1.27 (1.04, 1.57); p=0.02]. Tenofovir was detectable in 87 of 107 (81%) plasma samples available from a subset of 24 cases [median tenofovir plasma concentration: 83 ng/mL (IQR, 12.7-98.7)]. Overall, we identified a single person with concurrent proximal tubulopathy and ≥25% eGFR decline that clinically would be characterized as Fanconi syndrome. The participant was a 49 year old male in the FTC-TDF arm enrolled with CrCl 107 mL/min (serum creatinine 0.7 mg/dL), serum phosphorus 4.8 md/dL, trace dipstick proteinuria, and longstanding history of unspecified dermatitis.

Discussion

In this placebo-controlled study of daily oral FTC-TDF PrEP, TDF was not significantly associated with subclinical proximal tubular dysfunction over up to 24 months of observation. These results did not change in sensitivity analyses with alternate definitions for tubulopathy. We observed a higher frequency of isolated tubular proteinuria and isolated hyperuricosuria in persons on FTC-TDF compared to placebo. Because total proteinuria and albuminuria have been associated with increased risk of adverse outcomes, further research is needed to determine the clinical significance of non-albumin proteinuria. Our study has several strengths, including a prospective, randomized design with high adherence to PrEP [55,56], a placebo comparison, and a large sample size of men and women across a broad range of ages, and thus provides robust evidence assessing the effect of TDF-based PrEP on proximal tubular function in HIV-uninfected persons. The Partners PrEP study had the highest adherence to PrEP

of any reported PrEP trial, with tenofovir detectable in 82% of a random samples of participants; in the current analysis, plasma tenofovir concentrations were indicative of consistent daily dosing in the majority of included participants. The current analysis further enriched for drug exposure by including participants with 24 months of observation, making this cohort an important source of empirical evidence for TDF-related nephrotoxicity in HIV-uninfected persons [66].

An important concern for TDF use in healthy HIV-uninfected persons is the potential to cause kidney toxicity. Recent secondary safety analyses from the Partners PrEP and iPrEx studies reported that TDF-based PrEP is not associated with severe eGFR decline, but rather, a very small and non-progressive eGFR decline [57,62]. However, the absence of severe GFR decline does not preclude tubular injury [58]. The present study demonstrates that proximal tubular dysfunction is rare among healthy HIV-uninfected men and women with high adherence to daily oral FTC-TDF PrEP. The US Centers for Disease Control and Prevention and the World Health Organization have issued guidelines recommending PrEP be offered as a prevention option to persons at substantial risk for HIV acquisition [67,68]; our findings are informative for evidence-based guidelines for renal monitoring during PrEP use .

As previously reported [62], 69 (2%) participants on active PrEP developed a ≥25% eGFR decline in the Partners PrEP Study cohort. Whether early TDF-related proximal tubular dysfunction can be used to identify a minority of patients at increased risk of TDF-related Fanconi syndrome or progressive GFR decline has not previously been studied.

We found that a clinically relevant decline in eGFR (i.e. ≥25% eGFR decline from baseline) did not significantly correlate with proximal tubular dysfunction, using either a composite definition or individual markers of proximal tubular dysfunction. These findings suggest that monitoring with routine urine markers of proximal tubulopathy will not be an efficient approach to predict this rare but serious adverse renal event with PrEP. However, it is notable that the odds ratios for several individual markers of proximal tubulopathy were >2, suggesting the possibility that there is an association with eGFR decline but that proximal tubulopathy was not fully established at the earlier time point. Because it is unlikely that more definitive data will become available to address this possibility, it seems prudent to consider increased frequency of toxicity monitoring in persons with evidence of proximal tubulopathy based on serum or urine markers. We observed a single case of concurrent proximal tubulopathy and ≥25% eGFR decline that would be clinically characterized as Fanconi syndrome in a participant assigned to FTC-TDF PrEP in combination with potentially nephrotoxic co-medications, reinforcing the importance of toxicity monitoring and further studies in individuals at increased risk of adverse events. Importantly, this adverse event was also identified during routine follow-up in the Partners PrEP Study, and the abnormalities resolved rapidly after drug discontinuation.

Our data complement the findings of an optional substudy of iPrEx, which demonstrated a very low rate of proximal tubulopathy in men predominantly enrolled in South America [57]. The current analysis expands on that study by including both men and women with high TDF exposure and by preserving the benefits of randomization.

This study has several limitations. First, it is possible that the criterion used for characterizing proximal tubular dysfunction may be less sensitive than proposed biomarkers like retinol binding protein and β2-microglobulin. However, these biomarkers are more costly and data on their clinical usefulness in HIV-uninfected persons are limited. The tubular parameters used in this study are routinely available for clinical practice and have been used in prior studies. Second, tubular parameters were assessed at a single on-treatment visit without testing at baseline, thus incident tubulopathy could not be accurately characterized. Importantly, because our approach preserved randomized assignment, the interpretation of the between-group comparison is unaffected. Third, the parent trial excluded persons with baseline CrCl <60 mL/min, confirmed dipstick proteinuria, or concomitant use of nephrotoxic medications; consequently tubular function among those subpopulations could not be evaluated. In addition, the median age in our cohort was 36 years, and <10% of participants were age 50 years or older. Fourth, against a very low background rate of proximal tubulopathy, we had the ability to detect only large increases in the risk of proximal tubulopathy. Lastly, long-term PrEP effects beyond the study duration cannot be ascertained.

In conclusion, in this large placebo-controlled investigation, daily oral FTC-TDF PrEP was not associated with increased prevalence of proximal tubular dysfunction after up to 24 months of tenofovir use. The observation of a single case of overt Fanconi syndrome on active PrEP reinforces the importance of toxicity monitoring, particularly in individuals with risk factors for kidney injury, including concomitant nephrotoxic medications, older age, and comorbid risk factors for kidney disease. These findings support the safety of

TDF-based PrEP for up to 24 months as a component of HIV prevention in healthy HIV-uninfected individuals.

Conflict of Interest

CMW institution has received research grant funding related to tenofovir disoproxil fumarate from the National Institutes of Health and Gilead Sciences. JMB and CC have received research grants related to tenofovir disoproxil fumarate from US Government agencies and the Bill & Melinda Gates Foundation. Study medication for the Partners PrEP Study was donated by Gilead Sciences. All other authors declare no conflict of interest.

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Disclaimers: The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The views expressed are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Correspondence and request for reprints to: Dr. Kenneth Mugwanya; Address: 325 Ninth Avenue UW Box 359927 Seattle, WA 98104 Phone: (206) 520-3806 Fax: (206) 520-3831. Email: mugwanya@uw.edu

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Partners PrEP Study Team: *University of Washington Coordinating Center and Central Laboratories, Seattle, USA:* Connie Celum (principal investigator, protocol co-chair), Jared M. Baeten (medical director, protocol co-chair), Deborah Donnell (protocol statistician), Robert W. Coombs, Lisa Frenkel, Craig W. Hendrix, Jairam R. Lingappa, M. Juliana McElrath.

Study sites and site principal investigators: Eldoret, Kenya (Moi University, Indiana University): Kenneth H. Fife, Edwin Were; Kabwohe, Uganda (Kabwohe Clinical Research Center): Elioda Tumwesigye; Jinja, Uganda (Makerere University, University of Washington): Patrick Ndase, Elly Katabira; Kampala, Uganda (Makerere University): Elly Katabira, Allan Ronald; Kisumu, Kenya (Kenya Medical Research Institute, University of California San Francisco): Elizabeth Bukusi, Craig R. Cohen; Mbale, Uganda (The AIDS Support Organization, CDC-Uganda): Jonathan Wangisi, James D. Campbell, Jordan W. Tappero; Nairobi, Kenya (University of Nairobi, University of Washington): James Kiarie, Carey Farquhar, Grace John-Stewart; Thika, Kenya (University of Nairobi, University of Washington): Nelly R. Mugo; Tororo, Uganda (CDC-Uganda, The AIDS Support Organization): James D. Campbell, Jordan W. Tappero, Jonathan Wangisi.

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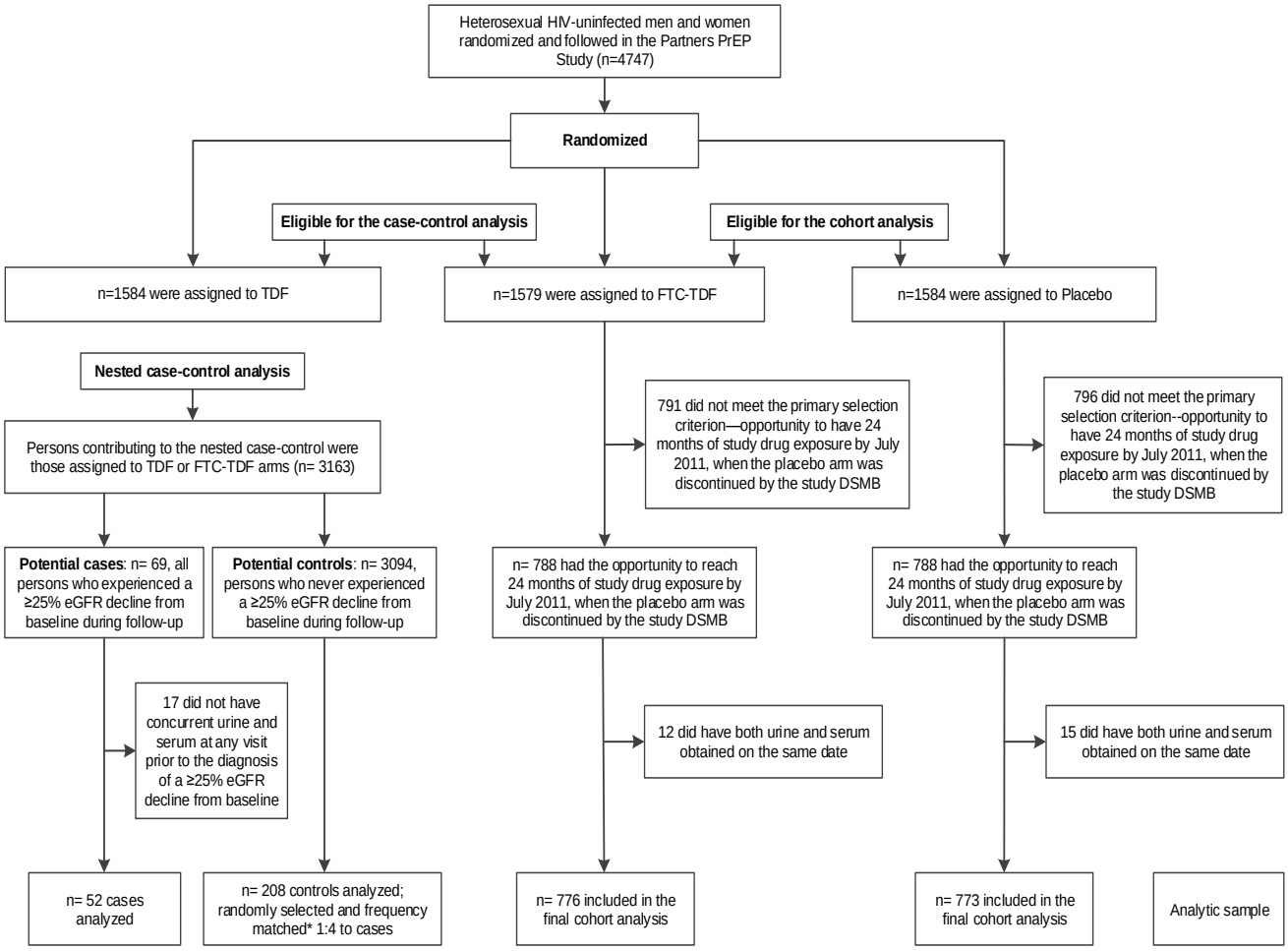

Figure 0:1. Study flow and participant selection

We used two complementary designs to answer two related questions; 1) a cohort analysis to determine whether FTC-TDF PrEP causes proximal tubulopathy, and 2) a nested case-control analysis of participants on either FTC-TDF or TDF PrEP to investigate whether proximal tubulopathy predicts a subsequent ≥25% eGFR decline from decline. The cohort analysis considered persons randomized to FTC-TDF and placebo in the Partners PrEP Study. The primary selection criterion for inclusion in the cohort analysis was the opportunity to have 24 month visit by July 10, 2011 when the study placebo arm was suspended by the DSMB; this criterion is based on a baseline variable "date of enrollment into the study" and thus preserves the randomized group assignment. Persons selected into the cohort approach (n=1549) tended to be of female sex, older age, with lower CrCl, and elevated blood pressure at baseline — characteristics for high propensity to experience kidney toxicity — compared to those not selected (n=1614). The nested case-control analysis considered persons assigned to active PrEP arms (FTC-TDF or TDF) who experienced a ≥25% eGFR decline ("cases"). Controls were persons on active PrEP who never experienced ≥25% decline in eGFR and where frequency matched to cases on study arm (either TDF or FTC-TDF) and duration of drug exposure.

eGFR, estimated glomerular filtration rate; PrEP, pre-exposure prophylaxis; FTC, emtricitabine; TDF, tenovfovir disoproxil fumarate; DSMB, Data and Safety Monitoring Board.

Table 0:1. Participant enrollment characteristics: Cohort analysis

Characteristics	*FTC-TDF (n=776)*	*Placebo (n=773)*	
	Mean ± SD or %	*Mean ± SD or %*	*P-value*
Male	65%	63%	0.39
Age-years	37 ± 9	37 ± 9	0.93
<35	43%	45%	
35-44	36%	35%	
≥45	21%	20%	
Creatinine Clearance –mL/minute	107 ± 24	107 ± 25	0.75
Serum Creatinine –mg/dL	0.79 ± 15	0.78 ± 15	0.29
Serum Bicarbonate –mEq/L	24.4 ± 3	24.2 ± 2.9	0.02
Serum Phosphorous –mg/dL	3.4 ± 0.7	3.4 ± 0.68	0.40
Elevated systolic blood pressure >140mmHg	3%	5%	0.29
Elevated diastolic blood pressure >90 mmHg	3%	4%	0.50
BMI categories (kg/m^2)	22.2 ± 3.3	22.3 ± 3.7	0.30
<18.5	9%	7%	
18.5-24.9	75%	75%	
25-29.9	13%	14%	
≥30	3%	4%	

Unless stated, column percent displayed. P-value is from Mann-Whitney-Wilcoxon test for continuous outcomes and chi-square or fisher exact testing for categorical variable where appropriate, testing the null hypothesis that the two distributions are identical. FTC, Emtricitabine; TDF, Tenovfovir disoproxil fumarate.

Table 0:2. Participant enrollment characteristics: Nested case-control analysis

	Cases (n=52)	Controls (n=208)	
Characteristics	*Median (range)*	*Median (range)*	p-value
Male	40%	68%	<0.01
Age-years	38 (19-58)	34 (18-58)	0.01
<35	35%	55%	
35-44	44%	33%	
≥45	21%	12%	
Creatinine Clearance –mL/minute	100 (60-162)	111 (60-172)	<0.01
Serum Creatinine –mg/dL	0.70 (0.49-1.06)	0.80 (0.40-1.24)	0.02
Serum Bicarbonate –mEq/L	24 (16-31)	24 (16-34)	0.79
Serum Phosphorous –mg/dL	3.4 (2.3-4.8)	3.4 (2.3-4.8)	0.35
Elevated systolic blood pressure >140mmHg	2%	3%	0.59
Elevated diastolic blood pressure >90 mmHg	2%	3%	0.70
BMI (kg/m^2) categories	22.4 (16.6-31.8)	22.2 (15.9-34.4)	0.96
<18.5	12%	10%	
18.5-24.9	69%	72%	
25-29.9	13%	14%	
≥30	6%	4%	
Months at time of sample collection	12 (3-24)	12 (3-24)	>0.99
Months at time of case diagnosis	15 (6-33)		

Unless stated, column percent displayed. P-value is from Mann-Whitney-Wilcoxon test for continuous outcomes and chi-square or fisher exact testing for categorical variable where appropriate, testing the null hypothesis that the two distributions are identical. FTC, Emtricitabine; TDF, Tenovfovir disoproxil fumarate.

Table 0:3. Frequency of markers of proximal tubular dysfunction in cohort analysis comparing FTC-TDF to placebo

Exposure definition	FTC-TDF (n=776)	Placebo (n=773)	Odds ratio (95%CL)	p-value
Primary analysis: ≥ 2 markers of proximal tubular dysfunction				
Fractional tubular resorption of phosphate <82%, Urine glucose ≥10 mg/dL with serum glucose ≤126 mg/dL, Urine total protein: urine creatinine ratio >200 mg/g with urine albumin: total protein ratio <0.4, Fractional excretion of uric acid >15%	13 (1.7%)	10 (1.3%)	1.30 (0.52, 3.33)	0.68
Sensitivity analysis for composite outcome				
Fractional tubular resorption of phosphate <95% with serum phosphate <2.6 mg/dL, all other criteria as in the primary analysis.	9 (1.2%)	8 (1.0%)	1.12 (0.38, 3.36)	>0.99
TmP/GFR <0.8 mmol/L, all other criteria as in the primary analysis	15 (1.9%)	11 (1.4%)	1.36 (0.58, 3.31)	0.56
Fractional tubular resorption of phosphate <95% with serum phosphate <2.6 mg/dL, % resorption of glucose <100%, all other criteria as in the primary analysis	8 (1.0%)	5 (0.7%)	1.60 (0.45, 6.24)	0.58
Individual markers of proximal tubular dysfunction				
Phosphate Handling				
Fractional tubular resorption of phosphate <82%	20 (2.6%)	21 (2.7)	0.95 (0.51, 1.76)	0.86
Fractional tubular resorption of phosphate <95% with serum phosphate <2.6 mg/dL	14 (1.8%)	8 (1.0%)	1.76 (0.73, 4.21)	0.21
TmP/GFR <2.6 mg/dL	34 (4.4%)	33 (4.3%)	1.03 (0.63, 1.68)	0.91

Glucose resorption				
Urine glucose ≥10 mg/dL with serum glucose ≤126 mg/dL	84 (10.8%)	96 (12.4%)	0.86 (0.63, 1.17)	0.32
Fractional resorption of glucose <100%	10 (1.3%)	7 (0.9%)	1.43 (0.54, 3.77)	0.63
Proteinuria				
Tubular proteinuria---Urine total protein: urine creatinine >200 mg/g with urine albumin: total protein ratio <0.4	57 (7.3%)	31 (4.0%)	1.90 (1.21, 2.97)	<0.01
Urine total protein: urine creatinine >200 mg/g	62 (8.0%)	34 (4.4%)	1.89 (1.23, 2.90)	<0.01
Uric acid excretion				
Fractional excretion of uric acid >15%	27 (3.5%)	10 (1.3%)	2.27 (1.32, 5.72)	0.001

P-values are for unadjusted chi-square or fisher's exact test, where appropriate, for the randomized comparison of participants assigned FTC-TDF versus placebo. Multivariate analyses including sex, baseline age, eGFR, indicator for elevated systolic blood pressure, and body mass index yielded similar results.

TmP/GFR, ratio of maximum tubular phosphate resorption to glomerular filtration rate; FTC, emtricitabine; TDF, tenofovir disoproxil fumarate

Table 0:4. Nested case-control analysis of relationship between eGFR decline of ≥25% and antecedent proximal tubular dysfunction

Exposure definition	Cases (n=52)	Controls (n=208)	Odds ratio (95%CL)	p-value
Primary analysis: ≥ 2 markers of proximal tubular dysfunction				
Fractional tubular resorption of phosphate <82%, Urine glucose ≥10 mg/dL with serum glucose ≤126 mg/dL, Urine total protein: urine creatinine ratio >200 mg/g with urine albumin: total protein ratio <0.4, Fractional excretion of uric acid >15%	2 (3.8%)	3 (1.4%)	1.39 (0.10, 14.0)	>0.99
Sensitivity analysis for composite outcome				
Fractional tubular resorption of phosphate <95% with serum phosphate <2.6 mg/dL, all other criteria as in the primary analysis.	1 (1.9%)	5 (2.4%)	0.58 (0.01, 5.58)	>0.99
TmP/GFR <0.8 mmol/L, all other criteria as in the primary analysis	1 (1.9%)	5 (2.4%)	0.58 (0.01, 5.58)	>0.99
Fractional tubular resorption of phosphate <95% with serum phosphate <2.6 mg/dL, % resorption of glucose <100%, all other criteria as in the primary analysis	1 (1.9%)	2 (1.0%)	1.55 (0.02, 32.26)	>0.99
Individual markers of proximal tubular dysfunction				
Phosphate Handling				
Fractional tubular resorption of phosphate <82%	4 (7.7%)	3 (1.4%)	5.24 (0.74, 42.10)	0.11
Fractional tubular resorption of phosphate <95% with serum phosphate <2.6 mg/dL	3 (5.8%)	5 (2.4%)	2.65 (0.39, 14.43)	0.36

TmP/GFR <2.6 mg/dL	4 (7.7%)	6 (2.9%)	2.64 (0.53, 11.70)	0.27
Glucose resorption				
Urine glucose ≥10 mg/dL with serum glucose ≤126 mg/dL	8 (15.4%)	30 (14.4%)	1.11 (0.40, 2.82)	0.97
Fractional resorption of glucose <100%	2 (3.8%)	2 (1.0%)	4.61 (0.30, 70)	0.33
Proteinuria				
Tubular proteinuria---Urine total protein: urine creatinine >200 mg/g with urine albumin: total protein ratio <0.4	2 (3.8%)	13 (6.3%)	2.18 (0.43, 2201)	0.53
Urine total protein: urine creatinine >200 mg/g	2 (3.8%)	14 (6.7%)	0.44 (0.04, 2.20)	0.48
Uric acid excretion				
Fractional excretion of uric acid >15%	3 (5.8%)	3 (1.4%)	2.93 (0.34, 24.47)	0.41

Comparisons were adjusted for age, sex, and baseline eGFR; P-values are for fisher's exact test comparing the frequency of markers of proximal tubular dysfunction between cases versus controls. Cases were persons on active PrEP who experienced a ≥25% decline in eGFR from baseline. Controls were persons on active PrEP without ≥25% decline in eGFR, frequency matched to cases on study arm (either TDF or FTC-TDF) and duration of drug exposure.

eGFR, estimated glomerular filtration rate; TmP/GFR, ratio of maximum tubular phosphate resorption to glomerular filtration rate; FTC, emtricitabine; TDF, tenofovir disoproxil fumarate

Pre-exposure prophylaxis use by breastfeeding HIV-uninfected women: a short-term study of antiretroviral excretion in breastmilk and infant absorption

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Pre-exposure prophylaxis use by breastfeeding HIV-uninfected women: a short-term study of antiretroviral excretion in breastmilk and infant absorption

Kenneth K. Mugwanya, MBChB, MS [1, 2, 3]; Craig W. Hendrix, MD[4], Nelly Mugo, MBChB, MPH[2, 5], Mark Marzinke, PhD[4] ; Elly Katabira, MBChB[6]; Kenneth Ngure, MPH, PhD[2,7]; Nulu Semiyaga, MBChB, MS[8]; Grace John-Stewart, MD, PhD[1,2,9,10]; Timothy R. Muwonge, MBChB, MPH[8]; Gabriel Muthuri, MBChB, MSc[11]; Andy Stergachis, PhD[2,12], Connie L. Celum, MD, MPH[1,2,9]; Jared M. Baeten, MD, PhD[1,2,9]

From the Departments of [1]Epidemiology, [2]Global Health, [9]Medicine, [10]Pediatrics, and [12]Pharmacy University of Washington, Seattle, USA; [3]Division of Disease Control, School of Public Health, Makerere University, Kampala, Uganda; [4]Department of Medicine, John Hopkins University, Baltimore, USA; [5]Kenya Medical Research Institute, Nairobi, Kenya; [6]Department of Medicine, Makerere University, Kampala, Uganda ; [7]Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya; [8]Infectious Diseases Institute, Makerere University, Kampala, Uganda; [11]Partners in Health Research and Development, Thika, Kenya.

Correspondence to: Kenneth K. Mugwanya, University of Washington 325 Ninth Avenue HMC # 359927 Seattle, WA 98104 Phone: (206) 520-3806 Fax: (206) 520-3831 Email: mugwanya@uw.edu

Abstract

Background: As pre-exposure prophylaxis (PrEP) becomes more widely used in heterosexual populations, an important consideration is its safety in infants who are breastfed by women taking PrEP. We investigated whether tenofovir and emtricitabine are excreted into breast milk and then absorbed by the breastfeeding infant in clinically significant concentrations when used as PrEP by lactating women.

Methods and Findings: We conducted a prospective short-term, open-label study of daily oral emtricitabine-tenofovir disoproxil fumarate PrEP among 50 HIV-uninfected breastfeeding African mother-infant pairs between 1-24 weeks postpartum (ClinicalTrials.gov Identifier: NCT02776748). The primary goal was to quantify the steady-state concentrations of tenofovir and emtricitabine in infant plasma ingested via breastfeeding. PrEP was administered to women through daily directly observed therapy (DOT) for 10 consecutive days and then discontinued thereafter. Non-fasting peak and trough samples of maternal plasma and breast milk were obtained at drug concentration steady-states on day 7 and 10, and a single infant plasma sample on day 7. Peak blood and breast milk samples were obtained 1-2 hours after maternal DOT PrEP dose while maternal trough samples were obtained at the end of the PrEP dosing interval [i.e. 23 to 24 hours) after maternal DOT PrEP dose]. Tenofovir and emtricitabine concentrations were quantified using liquid chromatography-tandem mass spectrometry assays.

Of the 50 mother-infant pairs enrolled, 48% were ≤12 weeks and 52% were 12-24 weeks postpartum, and median maternal age was 25 years [interquartile range (IQR) 22-28]. During study follow-up, the median (IQR) daily reported frequency of infant breastfeeding

was 15 times (12 to 18) overall, 16 (14 to 19) for the ≤12 weeks and 14 (12 to 17) for the 13-24 weeks infant age groups. Overall, median (IQR) peak concentrations in breast milk were 3.2 ng/mL (2.3 to 4.7) for tenofovir and 212.5 ng/mL (140.0 to 405.0) for emtricitabine. Similarly, median (IQR) trough concentrations in breast milk were 3.3 ng/mL (2.3 to 4.4) for tenofovir and 183.0 ng/mL (113.0 to 250.0) for emtricitabine, reflecting trough to peak breast milk concentration ratio of 1.0 for tenofovir and 0.9 for emtricitabine, respectively. In infant plasma, tenofovir was unquantifiable in 46/49 samples (94%) but emtricitabine was detectable in 47/49 (96%) [median (IQR): 13.2 ng/mL (9.3 to 16.7)]. The estimated equivalent doses an infant would ingest daily from breastfeeding were 0.47 µg/kg (IQR 0.35 to 0.71) for tenofovir and 31.9 µg/kg (IQR 21.0 to 60.8) for emtricitabine, translating into a <0.01% and 0.5% relative dose when compared to the 6 mg/kg dose that is used for therapeutic treatment of infant HIV infection and for prevention of infant postnatal HIV infection and has not shown safety concerns. No serious adverse effects were recorded during study follow-up.

Key study limitation was that only a single infant sample was collected to minimize venipunctures for the children. However, maternal daily DOT and specimen collection at drug concentration steady-state provided an adequate approach to address the key research question. Importantly, there was minimal variation in breast milk concentrations of tenofovir and emtricitabine (the respective median trough to peak concentration ratio ~1) demonstrating that infants were exposed to consistent drug dosing via breast milk.

Conclusion: In this short-term study of daily directly observed oral PrEP in HIV-uninfected breastfeeding women, the estimated infant doses from breast milk and resultant infant plasma concentrations for tenofovir and emtricitabine were 12,500 and

>200-fold lower than the respective recommended infant therapeutic doses and tenofovir was not detected in 94% of infant plasma samples. These data suggest that PrEP can be safely used during breastfeeding with minimal infant drug exposure.

Introduction

Women in Africa are disproportionately affected by HIV with the greatest rates of new HIV infections among women in their child-bearing ages [69]. Pregnancy and early post-partum are periods of heightened HIV risk associated with up to two-fold increased HIV acquisition risk [17,70-72]. Moreover, vertical HIV transmission to the breastfeeding infant is a potential serious consequence of maternal acute HIV seroconversion[18]. Antiretroviral pre-exposure prophylaxis (PrEP) with emtricitabine (FTC)-tenofovir disoproxil fumarate (TDF) co-formulation or TDF alone is a highly effective strategy to reduce the risk of sexual acquisition of HIV in both men and women[1-4,7]. The recent approval of FTC-TDF PrEP by some regulatory authorities in Africa will accelerate PrEP roll-out in this region [11,12].

With expanded access to PrEP, women who are breastfeeding may be prescribed PrEP. However, only limited data are available to assess the safety of PrEP use during breastfeeding. Currently, the US Centers for Disease Control and Prevention guidelines for PrEP permit preconception use of PrEP after discussion of the risk–benefit balance involved but have identified the need for additional data on infant drug exposure and safety during maternal FTC-TDF PrEP use during pregnancy and postpartum breastfeeding [67,73]. We investigated whether tenofovir and emtricitabine are excreted into human milk and then absorbed by the breastfeeding infant in clinically significant concentrations when taken as PrEP by their lactating HIV-uninfected mothers.

Methods

Population and study design

This was a prospective, open-label, single-arm study of daily oral FTC-TDF PrEP among 50 HIV-uninfected lactating women and their breastfeeding infants conducted between January and June 2015 at two clinical research sites in Thika, Kenya and Kampala, Uganda (ClinicalTrials.gov Identifier: NCT02776748). Eligible mothers were HIV seronegative and breastfeeding a singleton healthy infant, of legal age to provide written informed consent, had adequate renal function defined by normal creatinine levels and estimated creatinine clearance ≥60 mL/min, and were not infected with hepatitis B virus. Eligible infants were HIV-uninfected, aged 1-24 weeks, born at term to eligible women, and had no serious infections or active clinically significant medical problems. Recruitment into the study was stratified by infant age, with half ≤12 and half 13 to 24 weeks, to allow assessment of PrEP pharmacokinetics in breast milk among newborns and infants ages 3-6 months. The study protocol was approved by the University of Washington Human Subjects Review Committee, the Uganda National Council of Science and Technology, the Uganda National Drug Authority, the Kenya Medical Research Institute Scientific and Ethics Unit, and the Kenyan Pharmacy and Poisons Board. All women provided written informed consent and infant's father also provided written permission to enroll the infant.

Study procedures

Consenting HIV-uninfected breastfeeding women were followed with daily directly observed oral (DOT) FTC-TDF PrEP administered at the study clinic for 10 consecutive

days. The 10-day schedule was chosen to attain drug concentration at steady state levels (estimated to be achieved after 5 half-lives), sufficient to address the research question while minimizing potential undue infant drug exposure. PrEP was discontinued thereafter and no medication was administered directly to infants. Co-formulated FTC and TDF were dosed at 200 mg daily and 300 mg daily, respectively; these doses are the standard doses for prevention and treatment of HIV infection. Maternal blood and breast milk samples were obtained concurrently (i.e., within 30 minutes of each other) regardless of the timing of food intake (i.e., non-fasting) on the 7^{th} and 10^{th} day. Peak samples were obtained 1-2 hours after the maternal DOT PrEP and trough samples were obtained at the end of the dosing interval (i.e., 23 to 24 hours after DOT PrEP dose). A single infant blood sample was obtained after the maternal 7^{th} DOT PrEP dose. All collected blood samples were centrifuged immediately at 2000 relative centrifugal force for 15 minutes at room temperature and blood plasma aliquoted into 1 ml cryovials immediately. Breast milk was obtained by manual expression by the women and aliquoted into 1 ml cryovials immediately. All blood and breast milk specimens were stored below -80°C until testing. During daily follow-up, mothers completed a short quantitative interview about infant wellbeing, breastfeeding patterns, adverse events, and concomitant medication use. Both the mother and infant were monitored for adverse effects and the severity of clinical symptoms were scored using the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events [74].

Laboratory analytic method

Tenofovir and emtricitabine concentrations in plasma were quantified via previously validated liquid chromatographic-tandem mass spectrometric (LC-MS/MS) methods at the

Clinical Pharmacology Analytical Laboratory at the Johns Hopkins University School of Medicine (16). Further, LC-MS/MS methods for tenofovir and emtricitabine quantification in whole breast milk were developed and validated in accordance with the recommendations included in the US Food and Drug Administration, Guidance for Industry, Bioanalytical Method Validation guidelines [75]. Briefly, tenofovir and emtricitabine were isolated from whole breast milk via protein precipitation and quantified from a blood plasma calibration curve. The lower limits of quantification (BLQ) for emtricitabine in plasma and breast milk were 0.31 ng/mL and 1 ng/mL, respectively; BLQs for emtricitabine in plasma and breast milk were 0.31 ng/mL and 5 ng/mL, respectively.

Quantification of infant drug exposure

The primary measure of infant drug exposure through maternal breast milk was the concentrations of tenofovir and emtricitabine in infant plasma. Secondary measures were: a) maternal plasma and whole breast milk tenofovir and emtricitabine concentrations; b) milk to maternal plasma concentration ratios (M/P); and c) infant plasma drug to milk concentration ratio. To contextualize the clinical significance of the measured drug concentrations, we estimated two additional infant indices. First, we calculated the infant drug dose received from breast milk per day (infant dose), computed as the product of breast milk tenofovir and emtricitabine concentrations and the estimated volume of breast milk consumed by infant daily. Daily amount of breast milk consumed by the infant was assumed to be 150 mL/kg/day, the standardized milk consumption of the mean milk intake of a fully breast-fed infant [76-78]. Second, we calculated infant dose fraction, the drug dose a fully breastfed infant would ingest from maternal milk as a fraction of the infant's therapeutic dose per body weight. This was computed from infant daily dose from breast

milk and the weight-adjusted recommended therapeutic pediatric doses as: infant dose fraction (%) = infant dose from breast milk *100/infant therapeutic dose [76,79]. The respective therapeutic doses for tenofovir and emtricitabine was considered to be 6 mg/kg; this dose was derived from published doses considered be effective for prevention of mother to child transmission of HIV for age equivalent populations and has not shown safety concerns [22,80,81]. All outcome measures were evaluated separately for tenofovir and emtricitabine concentrations, stratified by the timing of sample collection (i.e., trough or peak).

Statistical analysis

The primary outcome was the proportion of infants with detectable steady-state concentrations of tenofovir and emtricitabine in plasma, overall, and stratified by infant age (i.e., ≤12 weeks or 13-24 weeks). Data were summarized as medians and interquartile ranges (IQR) for continuous variables and proportions for categorical outcomes. For one peak maternal plasma record, the tenofovir concentration (1040.0 ng/mL) was out of the assay analytic range (0.31-1000.0 ng/mL). This record was imputed to the upper limit of the assay analytic range. For concentrations below the assay limit of detection, a value of one-half of the detection limit was used in summary calculations for continuous variables. The non-parametric test, Mann-Whiney *U* test was used to compare the distribution of infant peak concentrations, daily dose from milk, and drug exposure index between the two infant age strata. All analyses were conducted in SAS version 9.4, SAS Institute Inc., Cary, NC.

Results

Population characteristics and follow-up

Of the 50 mother-infant pairs enrolled, 24 (48%) infants were ≤12 weeks of age, media (IQR) weight at study entry was 5 kg (4.3-6.0) for the ≤12 weeks group and 6.6 kg (6.0-7.1) for the 13-24 weeks group. Infants were breastfed for median of 15 (IQR 12-18) times daily during the week prior to study participation (Table 0:1); median proportion of infant food intake derived breastfeeding (surrogate for exclusive feeding), in week prior to study entry was 100%. During study follow-up, the median (IQR) daily frequency of infant breastfeeding was 15 times (12 to 18) overall, 16 (14 to 19) for the ≤12 weeks and 14 (range 12 to 17) for the 13-24 weeks infant age groups.

Overall, 499 of 500 (>99%) daily DOT PrEP doses were taken by the mother and 439 of 450 (98%) expected samples for pharmacokinetic analysis were collected: 198 maternal plasma (98 for peak and 97 for trough); 195 breast milk (98 for peak and 97 for trough); and 49 infant plasma samples. Peak maternal blood, breast milk, and infant blood samples were obtained after a median (IQR) of 63 (60 to 68), 70 (65 to 77), and 80 (45 to 90) minutes after maternal DOT PrEP dose, respectively while maternal trough samples were obtained a median of 23 hours (IQR 23 to 24) from the previous maternal DOT PrEP dose.

Tenofovir and emtricitabine concentrations in maternal plasma and breast milk

In maternal plasma, tenofovir was detected at concentrations consistent with steady-state use, and breast milk tenofovir concentrations were considerably lower than maternal

plasma (Figure 0:1). The median (IQR) peak steady-state concentrations of tenofovir in maternal plasma and breast milk were 152.0 ng/mL (IQR 56.9 to 321.0) and 3. 2 ng/mL (2.3 to 4.7), respectively, resulting in median peak milk/plasma (M/P) ratio of 0.03 (0.01 to 0.05) (Table 0:2). Similarly, median (IQR) trough steady state concentrations of tenofovir in maternal plasma and breast milk were 51.9 ng/mL (IQR 40.7 to 59.6) and 3.3 ng/mL (2.3 to 4.4), respectively, representing trough median M/P ratio of 0.07 (IQR 0.05 to 0.08).

Maternal plasma emtricitabine concentrations were also consistent with steady-state use, and emtricitabine concentrations in breast milk were more similar to plasma concentrations than had been seen for tenofovir (Figure 0:2). The median (IQR) peak steady state concentrations of emtricitabine in maternal plasma and breast milk were 267.5 ng/mL (103.0 to 1370.0) and 212.5 ng/mL (140.0 to 405.0), respectively, representing median peak M/P ratio of 0.63 (0.31 to 1.43) (Table 3). Similarly, the median (IQR) trough steady state concentrations of emtricitabine in maternal plasma and breast milk were 84.4 ng/mL (68.5 to 99.7) and 183.0 ng/mL (113.0 to 250.0) respectively, representing median trough M/P ratio of 2.1 (IQR 1.67 to 2.81). Overall, in contrast to maternal plasma concentrations, there was less variability in concentration of both tenofovir and emtricitabine in breast milk; median trough to peak breast milk [median (range) trough to peak breast milk concentration ratio; 1.0 (0.7 to 1.3) for tenofovir and 0.8 (0.4 to 1.3) for emtricitabine].

Infant exposure to tenofovir and emtricitabine from maternal breast milk

Overall, after 7 consecutive maternal daily DOT FTC-TDF PrEP, tenofovir was undetectable in 46 of 49 (94%) infant plasma samples; the three infants with detectable tenofovir also had detectable emtricitabine (Table 0:2). These three infants were aged 11, 13, and 17 weeks (plasma concentrations 0.9, 0.9, and 17.4 ng/mL, respectively and body weight 6.4, 5.8, and 6.2 kg, respectively) and their maternal milk tenofovir concentrations were modestly greater than the overall median (6.57, 3.64, and 4.05 ng/mL, respectively, versus median 3.2 ng/mL). There were no other notable unique characteristics between these three mother-infant pairs and the others. The median amount of tenofovir dose estimated to be ingested by an infant from breast milk was 0.47 µg/kg (IQR 0.35 to 0.71), translating into <0.01% (i.e. 12500-fold lower) of the proposed pediatric tenofovir therapeutic daily dose (6 mg/kg) [21,81]. Specifically, a 5-kg body weight infant would be expected to ingest a total tenofovir dose of 2.35×10^{-3} mg daily from breast milk compared to the proposed therapeutic daily dose of 30 mg.

Emtricitabine was detectable in 47 of 49 (96%) infant plasma samples (Table 0:3). The median (IQR) emtricitabine concentration in infant plasma was 13.2 ng/mL (9.3 to 16.7) overall, approximately 5% of breast milk concentrations: 16.6 ng/mL for infants aged ≤12-weeks and 10.5 ng/mL in infants 13-24 weeks. Based on the steady-state concentrations, the estimated median dose of emtricitabine expected to be ingested by the infant per day from breastfeeding was 31.9 µg/kg (IQR 21.0 to 60.8), translating into 0.5% (i.e. 200-fold lower) of the proposed pediatric emtricitabine therapeutic daily dose (6 mg/kg) [80,81]; the estimated doses were similar in the two infant age groups (Table 0:3). Specifically, a 5-kg body weight infant would ingest from breastfeeding a total daily emtricitabine dose of 0.16 mg compared to the recommended therapeutic dose of 30 mg per day.

Safety and tolerance

FTC-TDF was well tolerated by study mothers and infants. Over the 10-day maternal FTC-TDF PrEP dosing period, clinical symptoms recorded on ≥2 occasions were abdominal pain, diarrhea, and nausea in 3 (6%), 2 (4%), and 3 (6%) women, respectively (abdominal pain and nausea were concurrent in 2 women). In 2 infants (4%), diarrhea was reported on 2 visits during the study durations. These symptoms in both mother and infant were mild and resolved in 2-3 days. Of 50 women, 48 completed a safety kidney function screen at exit. Calculated creatinine clearance was >90 mL/minute at baseline and exit for all women [median: serum creatinine (0.64 vs 0.66 mg/dL) and creatinine clearance (107 vs 101 ml/min)].

Discussion

In this prospective study of daily directly observed doses of daily oral FTC-TDF PrEP in HIV-uninfected breastfeeding women, the estimated infant doses from breastfeeding and the resultant infant plasma concentrations for both tenofovir and emtricitabine were far below from what would result from the recommended pediatric doses. Based on breast milk concentration measurements, the estimated daily tenofovir and emtricitabine doses ingested by the infant through breast feeding were 12,500-fold and 200-fold, respectively, lower than the proposed daily pediatric dose for prophylaxis against vertical HIV acquisition. Thus, infants had low exposures to tenofovir and emtricitabine, which would not be expected to pose substantial safety risk to infants of mothers who use PrEP during breastfeeding.

Our study is the first to directly assess infant drug exposure via breast milk of mothers using FTC-TDF PrEP. We implemented an intensive daily maternal DOT PrEP dosing schedule to remove variability due to adherence. Daily oral PrEP offers an effective female-controlled option to reduce the risk of sexual HIV acquisition for women who are pregnant or breastfeeding, with the advantage relative to other prevention methods that it does not require cooperation of sexual partners. These data provide important empirical evidence to inform the discussion and assessment of risk to benefit balance of initiating or continuing PrEP during breastfeeding and are informative for evidence-based clinical guidelines. Although we were unable to implement a full concentration-time pharmacokinetic profile approach, our data collected at steady-state demonstrate minimal variation in the concentrations of tenofovir and emtricitabine in breast milk indicating that infants acquired consistent drug dosing throughout the day via breast milk. Thus, our findings suggest that PrEP can be safely used during breastfeeding with minimal infant exposure.

Our study provides both novel and complementary findings to the Agence Nationale de Recherche surle Sida (ANRS) 12109 [82], a pharmacokinetic study that assessed tenofovir and emtricitabine exposure in five HIV-infected Ivorian women dosed at 400 mg FTC–600mg TDF at the start of labor and 200 mg FTC– 300mg TDF daily for 7 days postpartum. In the 16 breast milk samples obtained in that study, simulated peak median infant tenofovir and emtricitabine daily doses from breast milk were 4.2 □g/kg and 146 □g/kg, respectively, which represented 0.03% and 2% of the respective therapeutic oral infant doses. Notably, these simulated infant doses are larger than what we found in this

study (estimated infant daily doses from breast milk: tenofovir =0.47 □g/kg and emtricitabine =31.9 □g/kg). One explanation is the difference in dosing and sampling schedules between the two studies. Alternatively, the difference could mean that infants are exposed to far smaller tenofovir and emtricitabine concentrations from breast milk based on direct plasma measure in our study than anticipated from the simulated doses in the ANRS study. Importantly, infant plasma drug concentrations were not directly measured in that study.

For breastfeeding women taking oral TDF, breast milk will exclusively contain tenofovir in an unesterified anionic form, and due to its poor oral bioavailability, negligible tenofovir concentrations would be expected to be absorbed by the infant from breastfeeding, consistent with our findings. In contrast, emtricitabine, which has excellent bioavailability, would be expected be absorbed to some degree from breast milk by the infant, as has been observed with structurally similar lamivudine and which was seen in this study. Although emtricitabine concentrations were quantified in the infant plasma, the concentration we observed in this study was a small fraction (~0.5%) of doses used in infant prophylactic daily doses to prevent vertical HIV acquisition.

For most drugs including tenofovir and emtricitabine, the dose below which there is no clinical effect in infants is unknown. A dose fraction index (exposure of 10% weight-adjusted therapeutic pediatric dose has been proposed as a safety threshold for infant exposure to maternal drugs from breast milk below which the degree of exposure to the drug in breast milk is considered clinically unimportant [83]. In this study, we found infant

plasma tenofovir and emtricitabine concentrations to be only <0.01% and 0.5% of the respective therapeutic pediatric doses. Accordingly, for TDF-based PrEP use during lactation, the small concentrations of tenofovir and emtricitabine absorbed by infant from maternal breast milk observed in our study are likely to be of limited clinical consequence.

Our results must be interpreted in light of the following limitations. First, we only tested for plasma tenofovir and emtricitabine concentrations, and not their pharmacologically active intracellular derivatives, tenofovir-triphosphate and emtricitabine-diphosphate concentrations, respectively. Second, we collected only a single infant sample to minimize venipunctures for the child. In sparse data pharmacokinetic situations like our study, where the traditional full drug concentration-time profile approach is not applicable, daily DOT and a steady-state sampling provide an adequate approach to address our key research question. Importantly, there was minimal variation in the concentrations of tenofovir and emtricitabine in breast milk demonstrating that infants were exposed to consistent tenofovir and emtricitabine dosing throughout the day via breast milk. Third, quantifying the volume of milk intake was not feasible, so we used the standard assumption of 150 mL/kg/day breast milk intake of a fully fed infant. Fourth, we only tested peak and trough maternal concentrations, which limits the precision of our M/P estimates throughout a dosing interval.

In conclusion, in this prospective study among HIV-uninfected breastfeeding African women using DOT FTC-TDF PrEP, nursing infants were exposed to low tenofovir and emtricitabine concentrations from breastfeeding than pediatric therapeutic doses. These data provide evidence suggesting that PrEP can be safely used during breastfeeding,

which is informative for clinical guidelines for women who are at substantial risk of HIV during pregnancy and the post-partum period.

Disclaimers: The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Table 0:1. General characteristics

Characteristic	All Infants (n=50)	Infant age ≤12 weeks (n=24)	Infant age 13-24 weeks (n=26)
Infant age –weeks	13 (9-19)	9 (6-10)	19 (17-21)
Birth weight –kg	3.4 (3.0-3.5)	3.3 (3.0-3.7)	3.4 (2.8-3.5)
Infant weight at screening-kg	6.0 (5.0 to 6.7)	5 (4.3-6.0)	6.6 (6.0-7.1)
Maternal age –years	25 (22-28)	24 (22-28)	26 (22-28)
Infant length –cm	58 (55-61)	55 (52-58)	60 (58-62)
Average daily frequency of breastfeeding, past week	15 (12-18)	16 (8-25)	15 (6-20)
Median proportion of infant feed due to breastfeeding	100% (100-100)	100% (100-100)	100% (100-100)
Maternal creatinine clearance –mL/min	107 (93-120)	109 (95-120)	105 (93-119)
Maternal serum creatinine –mg/dL	0.64 (0.58-0.71)	0.60 (0.57-0.68)	0.66 (0.58-0.72)
Maternal AST	21 (19-24)	22 (19-24)	20 (19-24)
Maternal ALT	19 (14-23)	19 (14-23)	22 (15-27)

Statistics are median (interquartile range) for continuous covariates and percent for binary variables. ALT, Alanine transaminase; AST, Aspartate aminotransferase.

Table 0:2. Tenofovir concentrations and infant exposure

Variable	All Infants	Infant age ≤12 weeks	Infant age 13-24 weeks	p-value
*Peak**	***n=98***	***n=49***	***n=49***	
Maternal plasma concentration –ng/mL	152.0 (56.9 to 321.0)	140.5 (53.3 to 327.5)	165.5 (58.4 to 309.0)	
Breast milk concentration –ng/mL	3.2 (2.3 to 4.7)	3.8 (2.7 to 6.9)	2.9 (2.1 to 3.8)	
M/P concentration ratio	0.03 (0.01 to 0.05)	0.03 (0.02 to 0.07)	0.02 (0.01 to 0.04)	
Proportion of infant plasma samples with concentration below the lower limit of quantification†	94% (46/49)	96% (23/24)	92% (23/25)	
Infant daily dose from breast milk –µg/kg	0.47 (0.35 to 0.71)	0.57 (0.41 to 1.04)	0.44 (0.32 to 0.56)	0.06
Infant dose fraction‡	<0.01 % (<0.01 to 0.01)	<0.01 % (0 to 0.02)	<0.01 % (<0.01 to 0.01)	0.06
*Trough**	***n=97***	***n=48***	***n=49***	
Maternal plasma concentration –ng/mL	51.9 (40.7 to 59.6)	54.1 (45.7 to 62.3)	46.0 (39.4 to 57.2)	
Breast milk concentration –ng/mL	3.3 (2.3 to 4.4)	3.5 (2.3 to 6.8)	3.2 (2.3 to 3.8)	
M/P concentration ratio	0.07 (0.05 to 0.08)	0.07 (<0.01 to 0.31)	0.07 (<0.01 to 0.11)	
Infant daily dose from breast milk –µg/kg	0.49 (0.34 to 0.66)	0.52 (0.05 to 0.08)	0.49 (0.05 to 0.08)	0.11
Infant dose fraction‡	<0.01% (<0.01 to 0.01)	<0.01% (<0.01 to 0.01)	<0.01% (<0.01 to 0.01)	0.11

Unless stated statistics are median (interquartile range). n are for samples tested with each woman providing a maximum of two of respective record (i.e, one day 7 and another on day 10), while each infant provided one record.

*Peak maternal blood, breast milk, and infant blood samples were obtained after a median (IQR) of 63 (60 to 68), 70 (65 to 77), and 80 (45 to 90) minutes after maternal DOT PrEP dose, respectively while maternal trough samples were obtained a median of 23 hours (IQR 23 to 24) from the previous maternal DOT PrEP dose.

† n=49, a single infant sample was obtained. Only 3 of 49 infant plasma samples had detectable tenofovir concentration records with detectable tenofovir concentration out of 49 infant plasma samples (1 infant aged 11 and 13 weeks [both 0.9 ng/mL] and 17 weeks [17.4 ng/mL]. Lower limit of quantification was <0.31 ng/mL in plasma and <1 ng/mL in whole breast milk.

‡ Infant dose fraction, represents the daily amount of tenofovir dose an infant would be expected to ingest from breast milk as a percentage of the recommended therapeutic daily dose (6 mg/kg).

P-values are from Mann–Whitney U test testing the null hypothesis that the two infant age groups are from the same distribution.

M/P, milk to maternal plasma ratio; Lower limit of quantification was <0.31 ng/mL in plasma and <1 ng/mL in whole breast milk.

Table 0:3. Emtricitabine concentrations and infant exposure

Variable	All Infants	Infant age ≤12 weeks	Infant age 13-24 weeks	p-value
*Peak**	***n=98***	***n=49***	***n=49***	
Maternal plasma concentration –ng/mL	267.5 (103.0 to 1370.0)	236.5 (93.6 to 1380.0)	533.0 (115.0 to 1370.0)	
Breast milk concentration –ng/mL	212.5 (140.0 to 405.0)	208.0 (139.5 to 377.5)	215.0 (149.0 to 431.0)	
M/P concentration ratio	0.63 (0.31 to 1.43)	0.70 (0.31 to 1.76)	0.59 (0.31 to 1.14)	
Infant plasma concentration‡ –ng/mL	13.2 (9.3 to 16.7)	16.6 (13.2 to 20.9)	10.5 (7.1 to 13.2)	<0.01
Infant plasma/milk concentration ratio	0.05 (0.03 to 0.08)	0.07 (0.04 to 0.10)	0.05 (0.02 to 0.06)	0.12
Infant daily dose from breast milk –µg/kg	31.9 (21.0 to 60.8)	31.2 (20.9 to 56.6)	32.3 (22.4 to 64.7)	0.94
Infant dose fraction†	0.5% (0.3 to 1.0)	0.5% (0.3 to 0.9)	0.5% (0.4 to 1.1)	0.94
*Trough**	***n=97***	***n=48***	***n=49***	
Maternal plasma concentration –ng/mL	84.4 (68.5 to 99.7)	82.8 (69.3 to 101.0)	84.8 (68.2 to 97.5)	
Breast milk concentration –ng/mL	183.0 (113.0 to 250.0)	187.5 (95.6 to 256.0)	183.0 (125.0 to 250.0)	
M/P concentration ratio	2.10 (1.67 to 2.81)	2.36 (1.48 to 2.83)	2.08 (1.69 to 2.81)	
Infant daily dose from breast milk –µg/kg	27.5 (17.0 to 37.5)	28.1 (14.3 to 38.4)	27.5 (18.9 to 37.5)	0.58
Infant dose fraction†	0.5 % (0.3 to 0.6)	0.5% (0.2 to 0.6)	0.5% (0.3 to 0.6)	0.58

Unless stated statistics are median (interquartile rage); n are for samples tested with each woman providing a maximum of two of respective record (i.e, one day 7 and another on day 10), while each infant provided one record.

*Peak maternal blood, breast milk, and infant blood samples were obtained after a median (IQR) of 63 (60 to 68), 70 (65 to 77), and 80 (45 to 90) minutes after maternal DOT PrEP dose, respectively while maternal trough samples were obtained a median of 23 hours (IQR 23 to 24) from the previous maternal DOT PrEP dose.

n=49, a single infant plasma sample was obtained. Emtricitabine was unquantifiable in only 2 of 49 infant plasma samples.

†Infant dose fraction (also called exposure index), represents the daily amount of emtricitabine dose an infant would ingest from breast milk as a percentage of the recommended pediatric therapeutic daily dose (6 mg/kg).

P-values are from Mann–Whitney U test testing the null hypothesis that the two infant age groups are from the same distribution.

M/P milk to maternal plasma ratio; Lower limit of quantification was <0.31 ng/mL in plasma and <5 ng/mL in whole breast milk.

Figure legend

Box plot of maternal and infant drug concentrations.

Non fasting maternal blood and breast milk samples were obtained concurrently (i.e., within 30 minutes) at the 7th and 10th visit (corresponding to 7th and 10th maternal DOT PrEP dose). A single infant blood sample was obtained after the maternal 7th DOT PrEP dose. Peak maternal blood, breast milk, and infant blood samples were obtained a median (IQR) of 63 (60 to 68), 70 (65 to 77), and 80 (45 to 90) minutes after the maternal DOT PrEP dose, respectively. Trough samples were obtained at close of the dosing interval, a median of 23 hours (IQR range 23 to 24) after the previous maternal Dot PrEP.

Middle box line represents the median.

Upper box line represents the 75th percentile and the lower box line represents the 25th percentile.

The top whisker denotes the maximum data value or the 3rd quartile plus 1.5 times the interquartile range, whichever is smaller.

The lower whisker denotes the minimum data value or the 3rd quartile plus 1.5 times the interquartile range, whichever is larger.

The notches display the 95% confidence interval around the median

Small circles represent outlier data points i.e., observations that are as extreme as ±1.5 of interquartile range.

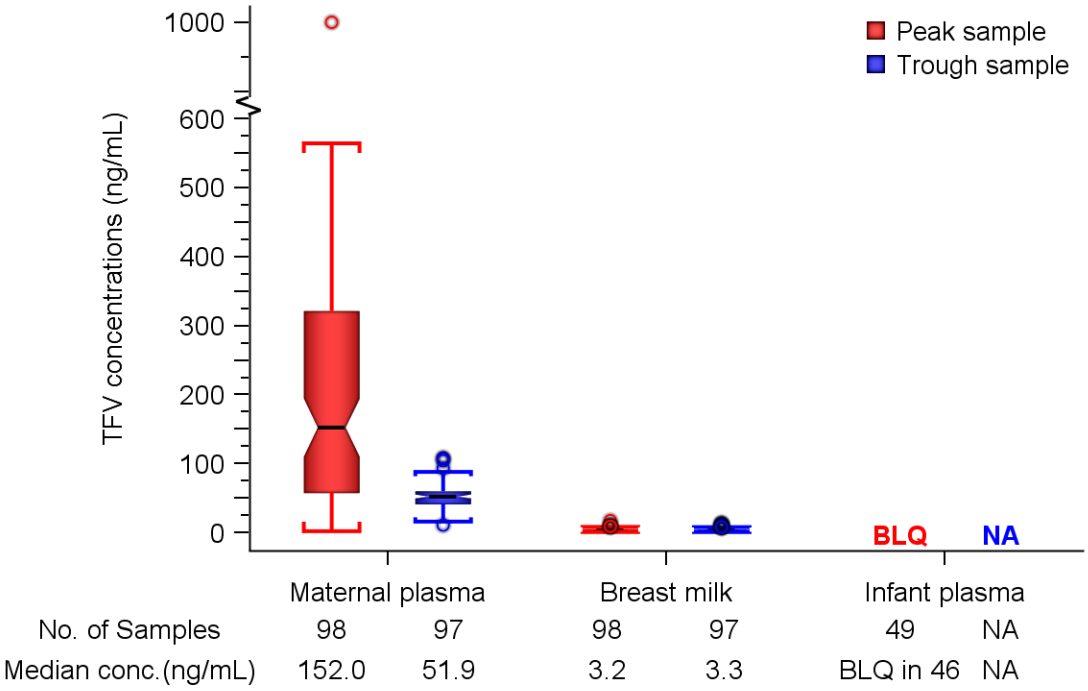

Figure 0:1. Maternal and infant tenofovir concentrations.
NA, not applicable, blq below limit of quantification (tenofovir: <0.31 ng/mL in plasma and <1 ng/mL in whole milk. Only 3 of 49 infant samples had quantifiable tenofovir concentration in plasma (infants aged 11 and 13 week s11 and 13 weeks [both 0.9 ng/mL] and 17 weeks [17.4 ng/mL]). For one outlier peak maternal plasma tenofovir concentration (1040 ng/ml) out of the assay analytic range (0.31-1000.0 ng/mL), the record was imputed to the upper limit of the assay analytic range.

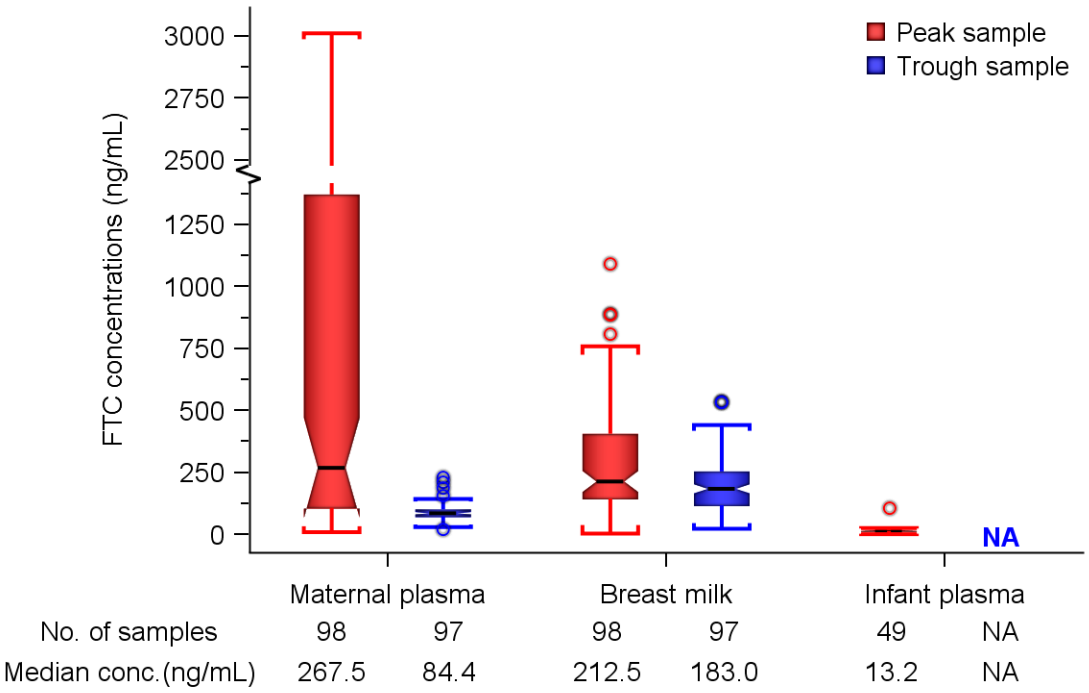

Figure 0:2. Maternal and infant emtricitabine concentrations.
NA, not applicable; lower limit of quantification: <0.31 ng/mL in plasma and <5 ng/mL in whole milk)

Sexual behavior of heterosexual men and women receiving antiretroviral pre-exposure prophylaxis for HIV prevention: a longitudinal analysis

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Sexual Behavior of Heterosexual Men and Women Receiving Antiretroviral Pre-Exposure Prophylaxis for HIV Prevention: A Longitudinal Analysis

Kenneth K. Mugwanya, MBChB [1, 2], Deborah Donnell, PhD [3, 4], Professor Connie Celum, MD [1, 4, 5], Katherine K. Thomas, MS[4], Patrick Ndase, MBChB[4], Nelly Mugo, MBChB[4, 6, 7], Professor Elly Katabira, MBChB[8], Kenneth Ngure, PhD[4, 7, 9], Jared M. Baeten, MD [1, 4, 5], for the Partners PrEP Study Team*

From the [1]Departments of Epidemiology, [4]Global Health, and [5]Medicine, University of Washington, Seattle, USA; [2]Department of Disease Control and Environmental Health, Makerere University, Kampala, Uganda; [3]Statistical Center for HIV/AIDS Research and Prevention, Fred Hutchinson Cancer Research Center, Seattle, USA; [6] Department of Obstetrics and Gynecology, University of Nairobi, Nairobi, Kenya; [7]Department of Obstetrics and Gynecology, Kenyatta National Hospital, Nairobi, Kenya; [8]Department of Medicine, Makerere University, Kampala, Uganda; [9]Institute of Tropical Medicine and Infectious Diseases, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya; * Members of the Partners PrEP Study Team listed after the Acknowledgements.

Short title: PrEP and sexual risk behavior

Correspondence to: Jared Baeten University of Washington 325 Ninth Avenue HMC # 359927 Seattle, WA 98104 Phone: (206) 520-3808 Fax: (206) 520-3831 Email: jbaeten@uw.edu

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Abstract

Background: Limited data are available to assess sexual behavior by persons using antiretroviral pre-exposure prophylaxis (PrEP) for HIV prevention. Increased sexual risk taking by persons using effective HIV prevention strategies, like PrEP, could offset HIV prevention benefits.

Methods: The Partners PrEP Study, a randomized, placebo-controlled trial of daily oral PrEP among heterosexual HIV-uninfected members of HIV serodiscordant couples, publicly reported efficacy for HIV prevention in July 2011 and participants continued monthly follow-up thereafter. We used regression analyses to compare the frequency of sex unprotected by a condom during the 12 months after compared to before July 2011 to assess whether knowledge of PrEP efficacy for HIV prevention resulted in increased sexual risk behavior.

Results: We analyzed 56,132 person-months from 3024 HIV-uninfected subjects (64% male). The average frequency of unprotected sex with the HIV-infected study partner was 59 per 100 person-months pre- versus 53 post-unblinding, reflecting no immediate change or change over time after July 2011 (p=0·66 and 0·25, respectively). There was a statistically significant increase in unprotected sex with outside partners over time after July 2011 but the effect was modest (average of 6.8 unprotected sex acts per year versus 6.2 acts in a predicted counterfactual scenario had unblinding not occurred, p=0·04). Compared to pre-July 2011, there was no significant increase in incident sexually transmitted infections or pregnancy after July 2011.

Interpretation: The transition from a blinded, placebo-controlled efficacy trial to all participants aware they were receiving active, efficacious PrEP in the Partners PrEP

Study provided a “natural experiment” to evaluate sexual risk compensation. PrEP, provided as part of a comprehensive prevention package, may not result in substantial changes in risk-taking sexual behavior for heterosexual couples. Our data are supportive of PrEP delivered as comprehensive combination HIV prevention package.

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Introduction

Three randomized trials have demonstrated that oral antiretroviral pre-exposure prophylaxis (PrEP) is efficacious in protecting against HIV acquisition in diverse geographic and at-risk populations[1-4]. Evidence of HIV prevention effectiveness for daily oral tenofovir-based PrEP, as well as for coitally-dependent tenofovir gel[84] and antiretroviral treatment as prevention,[85] has spurred optimism that the global HIV epidemic might be reversed. One important question for implementation of these prevention strategies following demonstration of effectiveness in trials is the potential for behavioral risk compensation, defined as persons using known effective HIV prevention interventions engaging in increased sexual risk-taking. A substantial increase in risky sexual behaviors by persons using PrEP, and other HIV prevention strategies, could offset the HIV protective benefits,[86] as well as increase the risk for sexually transmitted infections (STIs). In clinical trials of PrEP, there were no significant differences in sexual behavior between experimental and placebo groups;[1-4,15,16,87] however, because the comparison groups had equivalent uncertainty of treatment assignment and benefits of the study medication during the blinded trial period, absence of risk compensation may not fully reflect sexual behavior in the context of known PrEP efficacy.

In July 2011, the Partners PrEP Study, a randomized, double-blind, placebo-controlled trial of daily oral tenofovir (TDF) and emtricitabine(FTC)/TDF PrEP among HIV-uninfected members of African heterosexual HIV serodiscordant couples, demonstrated efficacy of PrEP for HIV prevention.[1] Participants who had been assigned to the active PrEP arms continued in the study and were informed they were receiving active PrEP and that PrEP had been demonstrated to reduce HIV acquisition risk. We examined

sexual behaviors before versus after July 2011 to assess the potential risk compensation after learning of the effectiveness of PrEP for HIV prevention. We hypothesized that individuals using PrEP who were aware of its proven efficacy against HIV acquisition might increase sexual behavioral risks.

Methods

Population

The Partners PrEP Study has been described previously (Clinicaltrials.gov number NCT00557245).[1,30] Briefly, between July 2008 and November 2010, 4747 HIV serodiscordant heterosexual couples were enrolled and followed at nine research sites in Kenya and Uganda. Eligible partners were ≥18 years of age, sexually active, and had normal hepatic and renal function. At enrollment, HIV-infected partners were not eligible for antiretroviral therapy, according to national guidelines.

HIV-uninfected partners were randomized in a 1:1:1 fashion to daily oral TDF, FTC/TDF, or placebo and followed monthly for up to 36 months with sexual behavioral assessment (questionnaire provided as online Appendix A), HIV serologic testing, pregnancy testing (for women), safety monitoring, risk-reduction counseling, and study drug provision. Laboratory testing for STIs (*Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and *Trichomonas vaginalis*) was done for all participants annually and when clinically indicated due to the presence of symptoms.

HIV prevention package and ethical review

All participants received a comprehensive package of HIV prevention services, which included HIV risk-reduction counseling (individually and as a couple), HIV testing, free condoms, testing and treatment for STIs, counseling and referral for male circumcision,

and, for HIV-infected partners, HIV primary care and referral for initiation of antiretroviral therapy according to national guidelines. The study protocol was approved by the University of Washington Human Subjects Review Committee and ethics review committees at each of the study sites. All participants provided written informed consent in English or their local language.

Data and Safety Monitoring Board (DSMB) review:

An independent DSMB met every six months to review the conduct of the placebo-controlled trial. At the July 10, 2011 meeting, the DSMB recommended that the placebo arm of the study be discontinued and the trial results be made public, due to definitive demonstration of PrEP protection against HIV acquisition. The primary results of the trial, using data through July 10, 2011, have subsequently been published.[1] Additionally, the DSMB recommended the active PrEP arms to be continued, to gain additional information on the relative efficacy, safety, and tolerability of PrEP using TDF versus FTC/TDF, and those receiving placebo to receive PrEP. Beginning on July 13, 2011, the study results were made public and research sites actively disseminated trial findings to study participants, through phone calls, group meetings, and at counseling sessions during their next scheduled monthly visits. Thus, continued follow-up of study participants initially assigned to the active PrEP arms provided an opportunity to evaluate risk behavior on open-label tenofovir-based PrEP after efficacy was announced. For subjects initially assigned to the active PrEP arms, study procedures were unchanged after July 13, 2011, with the exception of ongoing counseling about the efficacy of PrEP for HIV prevention.

For the present analysis, we considered data against a reference date of July 13, 2011 (Figure 0:1). Given that research sites required time to disseminate the trial results to all the study participants, we defined a "dissemination window" starting July 13, 2011 and including each participant's first subsequent study visit. A maximum of 12 monthly visits before and 12 visits after the dissemination window were included to provide an assessment of the effect of learning about the effectiveness of PrEP and being on active PrEP while minimizing temporal shifts in sexual behavior over periods of time greater than a year. All HIV-uninfected participants who were initially randomized to active PrEP were eligible for inclusion in the analysis. For participants initially assigned to the placebo arm, discontinuation and provision of active PrEP was done over a period of several months; because of this staggered gap during which no study procedures were conducted, participants on the placebo arm were not included in this analysis.

Outcome measurement

Four measures of sexual activity were explored: frequency of sex (vaginal or anal) without a condom (unprotected sex acts) and frequency of sex with or without a condom (total sex acts), with both their HIV-infected primary study partner (i.e., the partner with whom each subject enrolled into the study) and outside partners (i.e., any additional partner other than the primary study partner, including concurrent partners and partners acquired if the study partnership dissolved during follow-up).

Exposure measurement

The main predictor of interest was the participants' knowledge that they were receiving active PrEP and that PrEP had proven efficacy against HIV acquisition. We compared the blinded period (i.e., visits occurring before July 13, 2011) to the unblinded period (i.e., visits occurring after results dissemination window following July 13, 2011). Months at which PrEP was not dispensed, either due to a protocol-specified study drug hold (e.g., due to pregnancy or clinical adverse events) or a missed visit, were excluded to capture the direct effect of actual drug possession on sexual behavior.

Statistical Analysis

Crude frequencies were computed treating each visit as an independent observation. We used a segmented regression model,[88-90] fit for each count outcome variable using a zero-inflated negative binomial distribution.[91] The segmented model allowed for change in both the level (intercept, indicating an immediate change in behavior) and trend (slope, indicating a change over time) of the monthly frequency of sex acts before and following unblinding while controlling for potential secular changes (Figure 1). The zero-inflated negative binomial distribution allowed flexibility to account for unobserved heterogeneity and over-dispersion due to high occurrence of zeros common in sexual behavior data generated either as structural zeros (e.g. due to partnership break-up) or true sampling zeros. In our study, unprotected sex with HIV-infected partner was reported from only 13% of the scheduled study visits. The count and zero-model components of the zero-inflated negative binomial distribution were fit with identical covariates. Robust standard errors were used in all models to control for within person correlation.

Each model was specified with the following covariates: *time,* as a linear continuous variable in months since enrollment into the randomized trial to estimate the study background trend before July 13, 2011; *unblinding,* coded zero before and one after July 13, 2011, the main predictor of interest; and *time after unblinding,* as a linear continuous variable, coded zero before unblinding and 1-12 months after July 13, 2011, to estimate the change in trend after unblinding versus the study background trend. All models were adjusted for baseline sexual behavior, age, and gender. The model-predicted marginal means were used to compute annualized total frequency of sex acts estimated after unblinding and the counterfactual scenario that would have been expected had unblinding not occurred. The presented model estimates are interpreted conditional on the participant reporting being sexually active (i.e. not an always structural zero process).

In subgroup analysis, we evaluated the frequency of unprotected sex within the study HIV serodiscordant partnership by gender and in subpopulations with potentially high propensity for reproductive desires – those ≤30 years of age or who had no child with study partner – as these populations might be more likely to practice unprotected sex after receiving knowledge of PrEP efficacy for HIV prevention. As a sensitivity analysis, we repeated our primary analysis using shorter time periods: 3, 6, and 9 months pre- and post-unblinding.

Finally, as a cross-validation of self-reported sexual behavior, we compared the proportion of visits at which an STI (for all participants) and pregnancy (for female participants) were diagnosed during the two periods.

Reported P-values are two-sided for five percent type one error rate and were not adjusted for multiple comparisons. Analyses were conducted using SAS (version 9.2, SAS Institute) and Stata statistical software (version 12).

Role of the funding source

The authors designed and executed the study, had full access to the raw data, performed all analyses, wrote the manuscript, and had final responsibility for the decision to submit for publication. The funders had no role in design, data collection, analysis, interpretation, or writing of the manuscript.

Results

Study population

Of 4747 HIV-uninfected participants enrolled and followed in the Partners PrEP Study, 3163 were initially randomized to the clinical trial's active PrEP arms. Of these, 3024 were included in the present analysis; 139 were not included: 38 because they had seroconverted to HIV prior to July 13, 2011 and 101 because their final study visit (i.e., completing the protocol-specified 36 months of follow-up or early withdrawal) occurred on or prior to July 13, 2011. At enrollment, 64% were male, the median age was 34 years (interquartile range [IQR] 29 to 40), the median number of sex acts with the HIV-

infected study partner in the prior month was 4 (IQR 2 to 8), and 827 (27%) participants reported having at least one act of unprotected sex with their study partner in the prior month (Table 0:1). Before unblinding, participants had been followed for a median of 23 months (IQR 16 to 28).

A total of 60,406 person-months were accrued during the period for this analysis. After exclusion of months at which PrEP was not dispensed due to product holds or missed visits (n=4,274 months), the final analysis dataset included 56,132 person-months of observation: 33,198 pre-unblinding and 22,934 post-unblinding. Retention was similar during the two periods: 98% of expected visits were completed.

Frequency of sex with the HIV-infected study partner

The average crude frequency of unprotected sex with the HIV-infected study partner was 59 per 100 person-months pre-unblinding versus 53 post-unblinding (Table 0:2). There was a tendency toward a gradually decreasing trend in the frequency of unprotected sex during the study prior to unblinding (Figure 0:2A). After unblinding, there were no statistically significant changes in the immediate level (p=0·66) or trend (p=0·25) of unprotected sex (Table 0:2). The annual average total frequency of unprotected sex acts post-unblinding was 5·1 versus 4·9 that would have been expected in the counterfactual situation had unblinding not occurred.

Overall, the average frequency of total sex acts (i.e., both with and without condoms) with the HIV-infected study partner per 100 person-months was 414 pre- versus 361 post-unblinding (**Table 0:2.** Sexual frequency pre- and post-unblinding within and outside the primary study partnership). There was a tendency toward a decreasing trend in the frequency of total sex acts pre-unblinding (Figure 0:2B). After unblinding,

there were no statistically significant changes in the immediate level or trend in frequency of total sex acts (p=0·39 and 0·4, respectively). The estimated post-unblinding and counterfactual (i.e., predicted had unblinding not occurred) annual average total frequency of sex was not qualitatively different (42·4 versus 44·3, respectively).

Frequency of sex outside the primary study partnership

Overall, sex outside the primary partnership was reported at 12·4% (4,124/33,198, representing 794 individuals) of visits pre- versus 15·2% (3,480/22,934, representing 721 individuals) of visits post-unblinding. On average, the crude frequency of unprotected sex acts with outside partners per 100 person-months was 49 pre-unblinding versus 66 post-unblinding (Table 0:2). Before unblinding, there was a tendency toward an increasing trend in the frequency of unprotected sex with outside partners (Figure 0:3A). After unblinding, there was no immediate change in the level of unprotected sex (p=0·84). However, a modest but statistically significant increase in the frequency of unprotected sex over time was observed (p=0·04). The consequence of this change in trend was a small difference in the estimated versus counterfactual annual average total frequency of unprotected sex (6·8 vs. 6·2, respectively). Total sex act models with outside partners demonstrated qualitatively similar results (Table 0:2 and Figure 0:3B).

Sensitivity and subgroup analyses

Findings from the sensitivity analyses of shorter duration of months pre- and post-unblinding were consistent with that observed in the primary analyses (data not shown). In subgroup analyses, the level, trend, and the annualized estimated and counterfactual

cumulative frequency of unprotected sex with the HIV-infected partner were not substantially different during the two periods, except among the subgroup of men (Table 0:3). Among men, there was no immediate change in level for the frequency of unprotected sex acts (p= 0·61), but the frequency was modestly higher following unblinding (p-value for change in trend=0·04), with an estimated and counterfactual annual average total frequencies of unprotected sex of 5 vs. 4·9, respectively.

Finally, in cross-validation analyses, the proportions of visits (2467 visits pre- and 2768 post-unblinding with testing done) with diagnoses of STIs were similar before versus after-unblinding (p-values are for changes in immediate level and trend over time after unblinding): *N. gonorrhoeae* (1.0% of visits pre- versus 1·2% of visits post-unblinding, p=0·23 and p=0·62), *Chlamydia trachomatis* (1·1% versus 1·5%, p=0·11 and p=0·25], *Trichomonas vaginalis* (3·3% versus 2·9%, p=0·93 and p=0·56). Similarly, during 19,369 months of observation for women, incident pregnancy was detected at 125 of 11,611 (1·1%) months pre-unblinding versus 73 of 7758 (0·9%) months post-unblinding (p=0·21 and p=0·32 for changes in level and trend, respectively).

Discussion

The transition from a blinded, placebo-controlled phase to all participants aware they were receiving active, efficacious PrEP in the Partners PrEP Study provided a “natural experiment” to evaluate behavioral risk compensation in persons receiving open-label PrEP for HIV prevention. Our data suggest that providing PrEP as part of a comprehensive prevention package was not associated with substantial changes in

risk-taking sexual behavior, particularly within a known HIV serodiscordant partnership, over a period of up to 12 months of observation. Unblinding was associated with a small increase in the frequency of unprotected sex outside of the primary study partnership; however, this increase was not supported by clinical outcomes as neither STIs nor pregnancy were diagnosed more frequently after unblinding compared to before. The potential for risk compensation to undermine the protective benefits of current biomedical prevention technologies has been extensively discussed in the scientific and public domains,[24,25,92-94] although, the discussion related to PrEP has been largely hypothetical given the recent of demonstration of PrEP efficacy. To our knowledge, this study provides the first empirical data on sexual behavior in heterosexual persons receiving open-label oral PrEP for HIV prevention.

Prior studies have not demonstrated substantial behavioral risk compensation for other novel HIV prevention interventions, like medical male circumcision.[95,96] In the randomized, placebo-controlled trials of daily oral PrEP for HIV prevention, unprotected sex and STIs decreased after enrollment, in both the PrEP and placebo arms, suggesting that PrEP could be synergistic for risk-reduction when delivered along with a package of other HIV prevention services. Mathematical modeling suggests relatively little attenuation in population-level effectiveness of PrEP with doubling of risk behavior,[97,98] if PrEP has high efficacy and is taken with sufficient adherence to achieve efficacy. Thus, our data provide encouraging evidence that behavior changes as a result of PrEP may not undermine the public health benefits of PrEP.

Recent studies suggest that about a quarter of HIV infections in serodiscordant partnership occur from non-primary partners.[19] In a previous study of HIV-uninfected

members of serodiscordant couples, we found that sex with partners other than the HIV-infected study partner increased over time;[99] importantly, this generally reflected relationship dissolution with the original HIV serodiscordant partnership and new relationship formation rather than formal concurrency.[99] Similarly, in this study, average sexual frequency decreased over time with primary partners and increased with outside partners, and unprotected sex with outside partners was high among the minority of participants who reported sex outside the primary partnership. After unblinding, there was a small but statistically significant higher frequency of unprotected sex with outside partners; however, this did not translate into a substantial difference in the average annual total frequency of unprotected sex acts estimated after unblinding compared to the counterfactual that would have been expected without unblinding. For HIV serodiscordant couples, some partnerships dissolve, sometimes temporarily, and new partnerships are sometimes established, often with partners of unknown HIV serostatus with whom condoms may be used less than with known HIV seropositive partners. Effective messages regarding risk-reduction for concurrent and subsequent partners are needed to enhance counseling for HIV serodiscordant couples.

The ability to support a counterfactual inference in data collected over time is often threatened by alternative hypotheses including regression to the mean, maturation effects, and confounding. In absence of a nonequivalent control, use of multiple data points prior to the intervention can be useful.[100] In our study, we used up to 12 measurements prior to unblinding and separately modeled the trends pre- and post-unblinding to minimize the likelihood of potential maturation effects and secular changes that may have occurred even in the absence of the unblinding.

The results of this study must be viewed in light of its limitations. First, participants were couples experienced in research who received regular reinforcement of risk-reduction messages and had completed a median of 23 months of follow-up prior to unblinding. However, HIV serodiscordant couples in general are a priority group for HIV prevention and regular risk-reduction and adherence counseling will be part of a PrEP implementation package. Moreover, for this population, the background trend prior to unblinding was of decreasing risk behavior in the context of risk-reduction counseling. Second, the outcome measure, self-reported sexual behavior, is prone to reporting bias, but sensitivity analyses and cross-validation with incident STI and pregnancy data lend confidence to our findings. Third, we assumed a constant frequency and linear trend of sex acts in each segment, which was in general agreement with graphical presentations of the data. Despite these limitations, our study provides important new empirical evidence of the relationship between open-label use of PrEP and sexual behavior in heterosexual men and women. Given the large number of visits in our cohort and statistical efficiency gained from within-subject comparisons, our study was well powered to detect small differences in risky sexual behavior.

In conclusion, after unblinding of study participants, oral tenofovir-based PrEP was not associated with substantial risk-taking sexual behavior among heterosexual HIV-uninfected African men and women who continued PrEP. There was a modest increase in sexual risk-taking with outside partners, but no increase within known HIV serodiscordant relationships; importantly, there was no increase in clinical endpoints indicative of unprotected sexual activity. Ongoing counseling, including addressing HIV risks from concurrent and subsequent partners who may be of unknown HIV serostatus,

may help sustain risk-reduction for HIV-uninfected members of HIV serodiscordant couples using PrEP. Our data are supportive of PrEP delivered as comprehensive combination HIV prevention package.

Panel: **Research in context**

Systematic review

We searched PubMed for published studies through May 2013 assessing sexual behaviors of heterosexual persons using pre-exposure prophylaxis for HIV prevention.

Interpretation

To our knowledge, this study provides the first empirical data on sexual behavior in heterosexual persons receiving open-label oral pre-exposure prophylaxis, once the efficacy of pre-exposure prophylaxis for HIV prevention had been established in clinical trials. Our findings suggest that providing pre-exposure prophylaxis as part of a comprehensive prevention package may be not associated with substantial changes in risk-taking sexual behavior that would undermine the public health benefits of pre-exposure prophylaxis. HIV prevention programs that include pre-exposure prophylaxis should incorporate messages regarding risk-reduction, including for HIV serodiscordant couples, within and outside of the partnership.

Conflict of interest. None.

Author contribution

KKM, DD, CC, and JB conceived the design of the study. KKM and JB wrote the first draft of the manuscript. All authors contributed to the analysis, interpretation, and writing of the final manuscript. All authors have read and approved the final manuscript.

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Partners PrEP Study Team:

University of Washington Coordinating Center and Central Laboratories, Seattle, USA: Connie Celum (principal investigator, protocol co-chair), Jared M. Baeten (medical director, protocol co-chair), Deborah Donnell (protocol statistician), Robert W. Coombs, Lisa Frenkel, Craig W. Hendrix, Jairam R. Lingappa, M. Juliana McElrath.

Study sites and site principal investigators:

Eldoret, Kenya (Moi University, Indiana University): Kenneth H. Fife, Edwin Were; Kabwohe, Uganda (Kabwohe Clinical Research Center): Elioda Tumwesigye; Jinja, Uganda (Makerere University, University of Washington): Patrick Ndase, Elly Katabira; Kampala, Uganda (Makerere University): Elly Katabira, Allan Ronald; Kisumu, Kenya (Kenya Medical Research Institute, University of California San Francisco): Elizabeth Bukusi, Craig R. Cohen; Mbale, Uganda (The AIDS Support Organization, CDC-Uganda): Jonathan Wangisi, James D. Campbell, Jordan W. Tappero; Nairobi, Kenya (University of Nairobi, University of Washington): James Kiarie, Carey Farquhar, Grace John-Stewart; Thika, Kenya (University of Nairobi, University of Washington): Nelly R.

Mugo; Tororo, Uganda (CDC-Uganda, The AIDS Support Organization): James D. Campbell, Jordan W. Tappero, Jonathan Wangisi.

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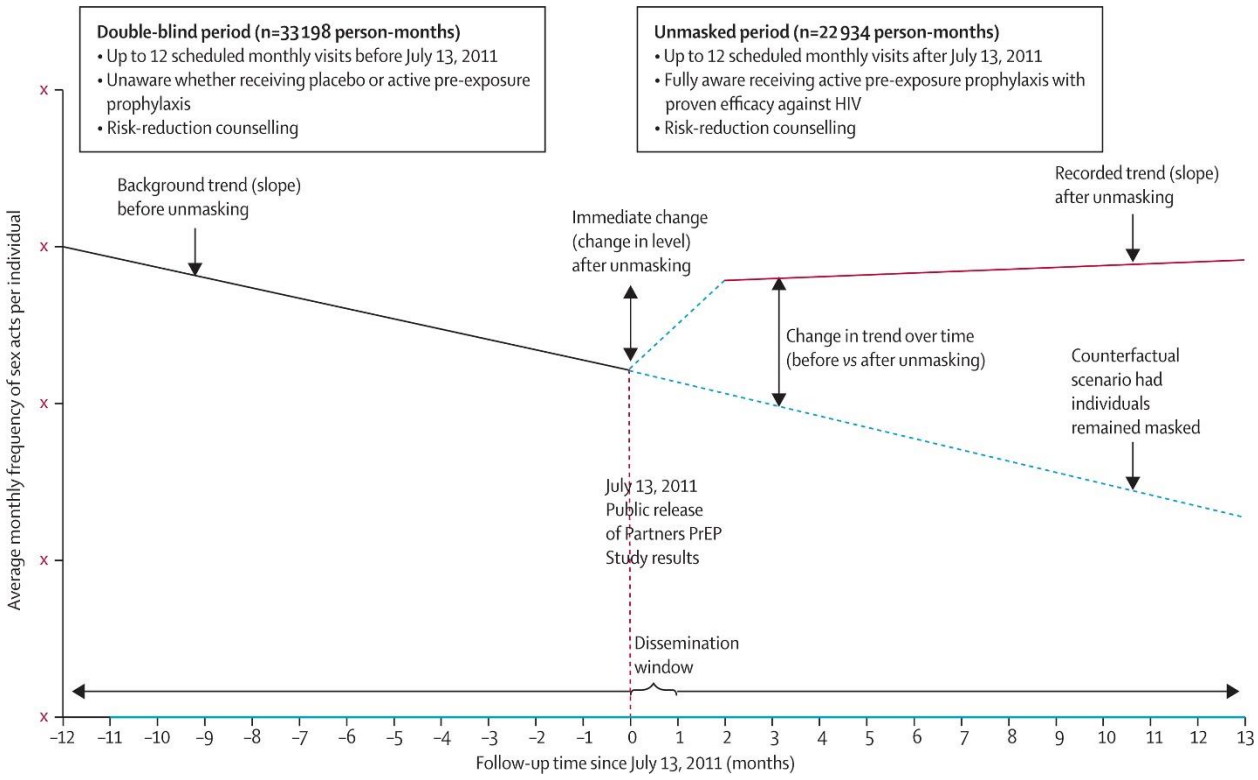

Figure 0:1. Schema of the study design and segmented regression analytic flow.

Represents a hypothetical segmented regression analytic flow. The study population provided data for up to 12 scheduled monthly visits both before and after July 13, 2011 when the Partners PrEP Study results were made public. The y-axis depicts the average frequency of sex acts per subject per month. Segmented regression analysis allowed estimation of the background trend of frequency of sex acts before July 13, 2011, change in level of the frequency of sex acts immediately following unblinding, and then the trend of the frequency of sex acts over time after unblinding. Interpretation of the results is based on change in the levels (immediate effect), changes in trend (trend after versus background trend) and predicted counterfactual frequency that would have been expected had unblinding not occurred.

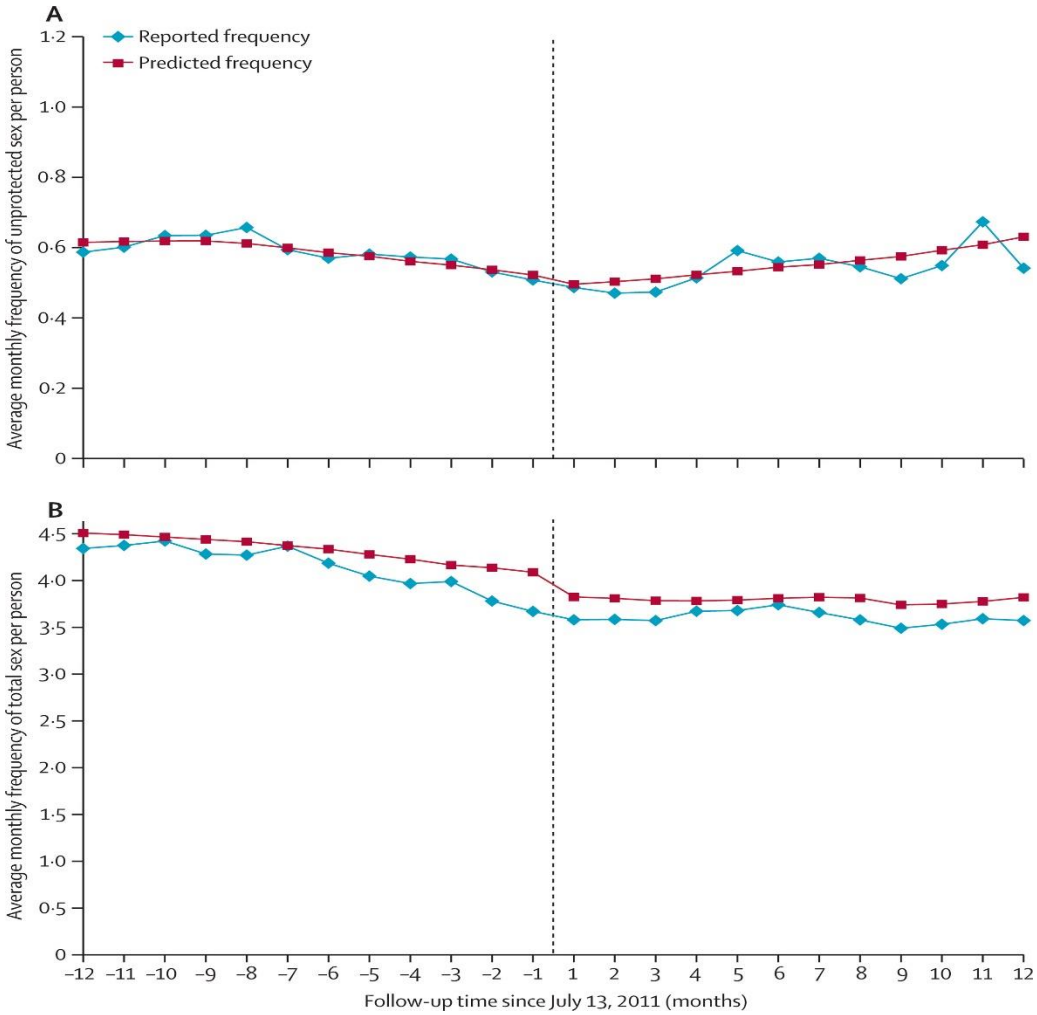

Figure 0:2. Trend of sex acts with HIV-infected study partner.
A, Trend of mean monthly frequency of unprotected sex acts with HIV-infected study partner per person before and after July 13, 2011. There was a tendency towards decreasing frequency with a significant trend before unblinding (p=0·03). No statistically significant changes in the level (p=0·66) and trend (p=0·25) of frequency of unprotected sex acts occurred following unblinding. Number of subjects at each visit (applies also to Figures 2B, 3A, and 3B): N=2507 at Month -12, N=2594 at Month -11, N=2680 at Month -10, N=2787 at Month -9, N=2839 at Month -8, N=2824 at Month -7, N=2818 at Month -6, N=2832 at Month -5, N=2838 at Month -4, N=2818 at Month -3, N=2818 at Month -2, N=2843 at Month -1, N=2638 at Month +1, N=2557 at Month +2, N=2470 at Month +3, N=2350 at Month +4, N=2209 at Month +5, N=1976 at Month +6, N=1785 at Month +7, N=1725 at Month +8, N=1581 at Month +9, N=1397 at Month +10, N=1249 at Month +11, and N=997 at Month +12.
B, Trend of mean monthly frequency of total sex acts with HIV-infected study partner per person before and after July 13, 2011. The pattern was that of decreasing trend (p=0·001) before unblinding, with no statistically significant changes in the level (p=0·39) and trend (p=0·4) of frequency of total sex acts following unblinding.

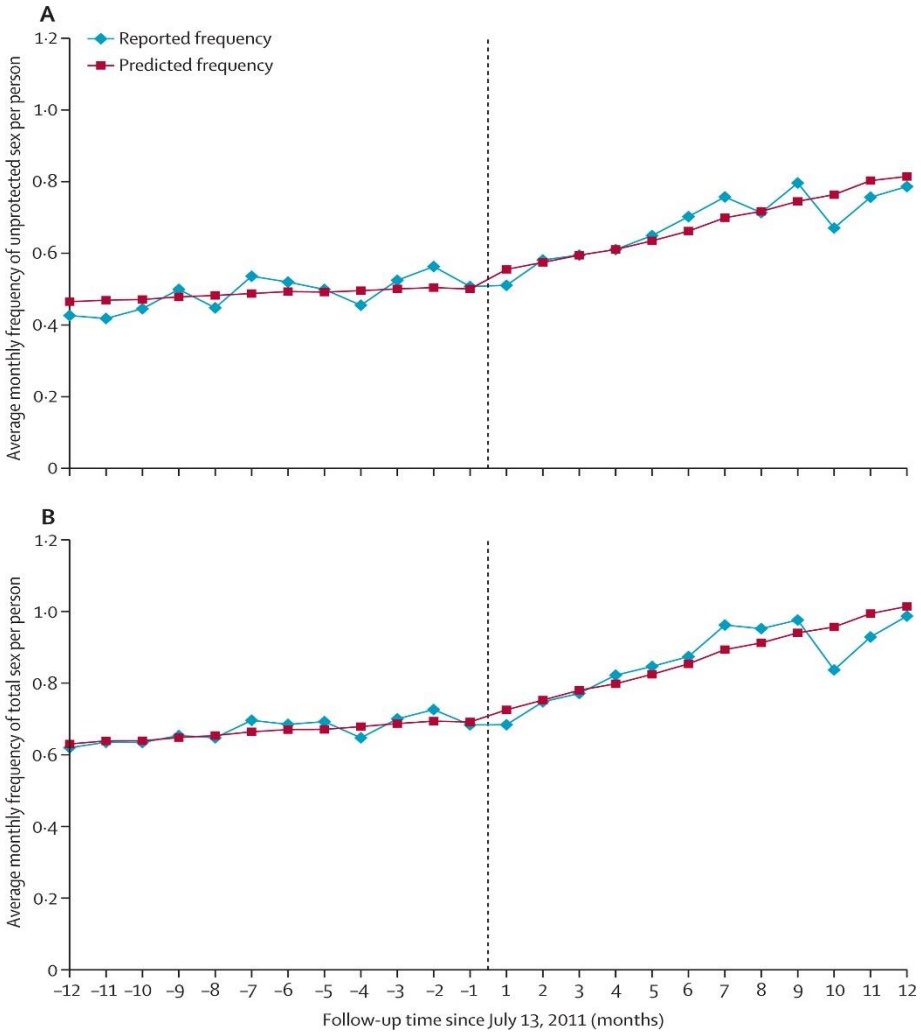

Figure 0:3. Trend of sex acts outside the primary study partnership
A, Trend of mean monthly frequency of unprotected sex acts per person outside the primary study partnership before and after July 13, 2011. Plots represent observed and predicted frequency of unprotected sex acts outside the primary study partnership with increasing trend before July 13, 2011. Following unblinding, the pattern remained that of an increasing trend but at a modestly faster rate compared to the background trend (p-value for change in trend=0·04).
B**,** Trend of mean monthly frequency of total sex acts per person outside the primary study partnership before and after July13, 2011. Plots represent observed and predicted frequency of total sex acts outside the primary study partnership with increasing trend before July 13, 211. Following unblinding, the pattern remained that of an increasing trend but at a modestly faster rate compared to the background trend (p-value for change in trend=0·006).

Table 0:1. Baseline characteristics of the study population.

Characteristics (n=3024)	**Median (interquartile range) or n (%)**
Male	1943 (64)
Age ≤30 years	1120 (37)
No child with study partner	683 (23)
Number of sex acts with HIV-infected study partner, prior month	4 (2-8)
Any unprotected sex with HIV-infected study partner, prior month	827 (27)
Any sex with partners other than the HIV-infected study partner, prior month	273 (9)
Any unprotected sex with partners other than the HIV-infected study partner, prior month	175 (6)

Table 0:2. Sexual frequency pre- and post-unblinding within and outside the primary study partnership

Characteristics	Crude average frequency of sex acts per 100 person-months* (95%CI)		Segmented model regression coefficients (β)ǂ¥ (95%CI)		Average cumulative number of sex acts in 12 months ǂ	
	Before unblinding	After unblinding	Immediate effect (change in level)	Effect over time (change in trend)	Counterfactual frequency ǂ	Estimated frequency after unblinding
Within the study primary partnership	59	53	-0·0304	0·0142	4.9	5.1
Unprotected sex acts	(58, 59)	(52, 54)	(-0·1660, 0·1050) P=0·66	(-0·0099, 0·0383) P=0·25		
Total sex acts	414	361	-0·0155	0·0026	44.3	42.4
	(411, 416)	(359, 363)	(-0·0511, 0·0200) P=0·39	(-0·0034, 0·0088) P=0·4		
Outside the primary partnership	49	66	0·0138	0·0204	6.2	6.8
Unprotected sex acts	(48, 49)	(65, 67)	(-0·1172, 0·1450) P=0·84	(0·0006, 0·0400) P=0·04		
Total sex acts	67	84	-0·0211	0·0247	8.8	9.0
	(66, 68)	(83, 85)	(-0·1362, 0·0939) P=0·72	(0·0071, 0·0424) P=0·006		

* Crude counts computed from independent monthly observations during each period from 3024 HIV seronegative partners.
ǂ Adjusted for within subject correlation, secular changes, age, gender, and baseline sexual behavior in month prior to enrollment in the trial.
¥ The beta coefficients represent differences in the month-to-month changes in the frequency of sex acts.
‡ Predicted frequency of sex acts that would have been expected in a counterfactual scenario if unblinding had not occurred.

Table 0:3. Subgroup comparisons of frequency of unprotected sex with the HIV infected study partner pre- and post-unblinding

Characteristics	Segmented model regression coefficients (β) ¥ * (95%CI)		Average cumulative number of sex acts in 12 months after unblinding*	
	Immediate effect (Change in level)	Effect over time (Change in trend)	Counterfactual frequency ǂ	Estimated frequency after unblinding
≤30 years	-0·0182 (-0·2416, 0·2051) P=0·87	0·0230 (-0·0193, 0·06-54) P=0·29	5.5	5.5
No child with study partner	-0·0558 (-0·3613, 0·2497) P=0·72	-0·0140 (-0·0665, 0·0385) P=0·60	5.2	5.2
Females	0·0037 (-0·2120, 0·2195) P=0·97	-0·0214 (-0·0645, 0·0216) P=0·33	4.9	5.2
Males	-0·0450 (-0·2197, 0·1296 P=0·61	0·0297 (0·0019, 0·0574) P=0·04	4.9	5.0

* Adjusted for within subject correlation, secular changes, age, gender, and baseline sexual behavior in the month prior to enrollment in the trial.

¥ The beta coefficients represent differences in the month-to-month changes in the frequency of sex acts.

ǂ Predicted frequency of sex acts that would have been expected in a counterfactual scenario if unblinding had not occurred.

A systematic review of safety of oral tenofovir disoproxil fumarate-based pre-exposure prophylaxis for HIV prevention

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A systematic review of Safety of Oral Tenofovir Disoproxil Fumarate-Based Pre-Exposure Prophylaxis for HIV Prevention

Kenneth K. Mugwanya[1, 2, 3] MBChB, MS; Jared M. Baeten[1, 2, 4], MD, PhD

Affiliations:
Departments of Epidemiology[1], Global Health[2], and Medicine[4] University of Washington, Seattle, USA; Division of disease control, school of Public Health[3] Makerere University, Kampala Uganda

Correspondence to: Jared Baeten, University of Washington 325 Ninth Avenue UW Box 359927 Seattle, WA 98104 Phone: (206) 520-3808 Fax: (206) 520-3831 Email: jbaeten@uw.edu

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Abstract

Introduction: Tenofovir disoproxil fumarate (TDF)-based pre-exposure prophylaxis is a novel HIV prevention strategy for individuals at increased sexual risk for HIV infection. For any biomedical prevention intervention, the bar for tolerating adverse effects in healthy persons is high compared to therapeutic interventions.

Areas covered: We provide a concise summary of the clinical safety of TDF-based pre-exposure prophylaxis with focus on TDF-related effects on tolerability and side effects, kidney function, bone density, HIV resistance, sexual and reproductive health. The evidence base for this review is derived from a literature search of both randomized and observational studies evaluating efficacy and safety of TDF-based PrEP, TDF alone or in combination with emtricitabine, identified from PUBMED and EMBASE electronic databases, clinicaltrials.gov and major HIV conferences.

Expert opinion: TDF-based pre-exposure prophylaxis is a potent intervention against HIV acquisition when taken which is generally safe and well tolerated. The risk of the small, non-progressive, and reversible decline in glomerular filtration rate and bone mineral density as well as the potential selection for drug resistance associated with PrEP are outweighed, at the population level and broadly for individuals, by PrEP's substantial reduction in the risk of HIV infection.

Introduction

Tenofovir disoproxil fumarate (TDF), alone or in combination with emtricitabine (FTC), reduces the risk of HIV acquisition in individuals at substantial risk of HIV-infection when used as pre-exposure prophylaxis (PrEP) [1-4]. FTC and TDF are widely prescribed as part of combination antiretroviral therapy for the treatment of HIV. While generally safe and well tolerated in HIV-infected persons, TDF is infrequently associated with increased risk of some toxicities, including renal impairment (ranging from mild to occasionally severe, with higher risk among persons of African descent vs Caucasians)[13,101] and loss of bone mineral density[102]. Moreover, use of antiretroviral medications, for treatment of HIV and potentially for prophylaxis as well, carries some risk of selection for HIV viruses harboring antiretroviral resistance. Thus, the use of TDF and FTC-TDF as PrEP has raised important questions about the safety of these medications for use as preventative agents. Importantly, for all biomedical prevention interventions, the bar for tolerating adverse effects must be high compared to therapeutic interventions, as the group using the preventative intervention is otherwise healthy and has only a chance of contracting the condition being prevented. For PrEP, given the epidemiology of HIV risk, the majority of persons who will be prioritized for implementation of PrEP will likely be younger, otherwise healthy, and with minimal use of concurrent medications[68]. In this article, we provide a concise review and summary of current data on the clinical safety of oral TDF-based PrEP for healthy adults for HIV prevention with focus on TDF-related tolerability and adverse effects.

Mechanism of action and clinical pharmacology

TDF is a prodrug for tenofovir (Table 0:1), an acyclic nucleotide analogue reverse transcriptase inhibitor[103]. Tenofovir is a potent competitive inhibitor of HIV and hepatitis B virus reverse transcriptase that is additive or synergistic when combined with other antiretroviral agents inhibiting viral replication[103]; for both HIV and HBV, it has a high barrier for the development of viral resistance mutations. It has a long elimination and intracellular half-life (~17 and >60 hours, respectively), allowing for once-daily dosing. The oral bioavailability of TDF in fasted subjects is ~25% and following absorption, TDF is rapidly (<1 minute) converted to tenofovir which is metabolized intracellularly to the active metabolite, tenofovir diphosphate[104]. Maximum tenofovir plasma concentrations are achieved within 1 to 2 hours of oral administration[104]. TDF (and FTC) is not an inducer or a substrate for cytochrome P450 enzymes but is primarily eliminated unchanged in urine by a combination of glomerular filtration and active proximal tubular secretion[104]. About 20-30% of the tenofovir is actively transported across the basolateral membrane into the proximal tubular epithelial cells by organic anion transporters [105], with active efflux into the tubular lumen across the apical membrane via the multi-drug resistance proteins transporters[106], intrinsically making the proximal tubule epithelial cells a target for tenofovir-related kidney toxicity[107]. The pathogenesis of TDF-related toxicities is not well elucidated but may be a consequence of effects on the proximal tubule epithelial cell mitochondria[53,108], although tenofovir is a weak inhibitor of mammalian and mitochondrial DNA polymerases compared to the structurally similar acyclic nucleotide analogues, cidofovir and adefovir.

Clinical application of TDF for HIV prevention

PrEP involves administering antiretroviral medications to HIV-uninfected, at-risk individuals to lower their risk of sexual HIV acquisition. Biological plausibility to support efficacy and safety trials of antiretroviral PrEP against HIV acquisition was derived from human studies of post-exposure prophylaxis and of use of antiretrovirals for the prevention of perinatal HIV transmission; animal studies of vaginal and rectal viral challenge provided models of PrEP efficacy [109-113]. Randomized clinical trials have demonstrated that oral TDF-based pre-exposure prophylaxis is highly effective against HIV acquisition in a diverse at-risk populations and geographical regions, with protective effectiveness of 44-75% in the randomized comparisons and >90% in persons adherent to PrEP as prescribed. However, a lack of efficacy was observed in two PrEP trials in African women [15,16], thought to be due to very low adherence to PrEP in those studies. In 2012, the US Food and Drug Administration approved daily oral FTC-TDF for HIV prevention in persons with heightened risk for HIV in combination with other HIV prevention strategies[73]. Subsequently, the US Centers for Disease Control and Prevention has issued detailed guidelines for the delivery of PrEP in clinical settings[67], and, recently, the World Health Organization issued guidelines recommending PrEP be offered as a prevention options to persons at substantial risk for HIV acquisition[68,114]. TDF alone is also effective for HIV prevention. Recent evidence from pragmatic trials has demonstrated high protective effectiveness for PrEP against HIV infection in “real world” settings[5-7].

Safety evaluation

Evidence for this review was derived from a search of literature on PrEP efficacy and safety from randomized clinical trials, open-label extensions following randomized trials, implementation PrEP projects, and observational studies. Articles published in English between January 1, 1999 and August 15, 2015 were identified from PubMed and EMBASE electronic databases, and the clinical trials registry (www.clinicaltrials.gov) using combination search terms (preexposure prophylaxis, pre-exposure prophylaxis, PrEP, HIV, Truvada, tenofovir, antiretroviral, chemoprophylaxis, and toxicity category). Abstracts from major HIV conferences (e.g., Conference on Retroviruses and Opportunistic Infections, International AIDS Conference, and IAS Conference on HIV Pathogenesis, Treatment, and Prevention) were also considered. In addition, we reviewed reference lists of relevant papers. Toxicity signals are summarized under the following categories: general side effects, kidney, bone, HIV resistance, and sexual and reproductive health. The studies contributing to this review and the summary of evidence are provided in the Table 0:2.

General and any adverse effects

Overall, the frequency of any adverse events (i.e. any clinical or laboratory finding), both overall, grade 3/4, and serious adverse events, was no greater for persons assigned PrEP versus those assigned placebo or no PrEP in clinical trials[1-4,15,115-118]. General gastrointestinal symptoms, commonly referred to as PrEP start-up syndrome, (primarily nausea, but also including diarrhea, vomiting, and abdominal pain), occurred in 1- 18% of participants assigned active PrEP compared to 0-10% in persons assigned placebo.

PrEP start-up symptoms are generally limited to within the first month and end spontaneously[1-4,6,15,115,116,119].

Kidney toxicity

An important concern for the use of TDF for PrEP is the potential for kidney toxicity. Three secondary analyses of the Partners PrEP, iPrEx, and Bangkok Tenofovir studies provide detailed analyses of changes in estimated glomerular filtration rate (eGFR), a commonly-used measure of overall kidney function. In those studies, TDF-based PrEP was associated with a small but statistically significant decline in eGFR that was non-progressive and resolved with TDF discontinuation. In the Partners PrEP Study[62], with a median per-protocol follow-up of 18 months, declines in eGFR attributed to TDF-based PrEP versus placebo were 1-3 mL/min/1.73 m^2 ($p < 0.05$), a change of <1.5% from baseline that was non-progressive for 36 months and was not accompanied by a significant increase in the likelihood of a clinically relevant change in eGFR (i.e., ≥25% decline from baseline). Similar magnitude of decline in calculated creatinine clearance were observed in the iPrEx cohort after up to 81 weeks of observation (~2% loss in creatinine clearance from baseline) that was stable through the last on-treatment visit [57]. In the Bangkok Tenofovir Study[2,61], the net decline in creatinine clearance attributable to TDF versus placebo were 1-5 mL/min significantly lower for participants assigned TDF versus placebo up to 60 months. Overall, the frequency of graded creatinine elevations were more common in PrEP arms but not statistically different from placebo and the reported kidney abnormalities resolved after TDF was discontinued [1-4,15,16,57,61,62,115-118,120]. Detailed evaluation of proximal tubular function, was only reported by an optional

sub-study of the iPrEx trial among 1137 participants (563 FTC-TDF, 574 placebo)[57]. In that sub study, the frequency of abnormal fractional excretion of phosphorus and uric acid, glycosuria in presence of normal serum glucose, and proteinuria was rare and no more common in persons assigned FTC-TDF than placebo. Similarly, other studies reporting on the frequency of graded decreased phosphorus did not observe difference between TDF-based PrEP versus placebo.

Bone toxicity

Loss of bone mineral density (BMD) and potentially bone fractures is another concern for TDF-based PrEP, potentially as a consequence of TDF-related phosphate wasting[121]. Overall, TDF-based PrEP appears to be associated with modest (0.4% to 1.6%) but statistically significant net loss in BMD among HIV-uninfected individuals without elevation in the risk of bone fractures[1,2,4,16,122-124]. The observed decline resolves to baseline level after TDF is discontinued. In a sub-study of the iPrEx cohort (247 subjects receiving FTC-TDF, 251 placebo)[124], 12% and 2% of participants had low BMD in the spine and the hip at baseline, respectively, and FTC-TDF PrEP was associated with a net BMD decrease versus placebo at the spine or hip of -0.6% to -0.91% at 24 weeks that was stable up to 96 weeks with no elevated risk for bone fractures. BMD decline was correlated with tenofovir plasma levels. In a sub-study of CDC TDF Safety Study among HIV-negative men who have sex with men (94 on TDF, 90 on placebo)[123], baseline low BMD (z-score) was 10% and the net mean BMD decline for TDF versus placebo were -0.7% to -1.1% at femoral neck, total hip or at the L2–L4 spine over 24 months of follow-up; 13% of participants on TDF vs. 6% placebo of participants

experienced ≥5% BMD loss at the femoral neck at 24 months (p =0.13) with no significant difference in the risk for bone fractures (p =0.75). In a BMD sub-study of the TDF2 Study (68 on FTC-TDF, 79 on placebo)[122], net BMD decrease from baseline at forearm, spine, and hip was 0.8% to 1.6% lower for TDF-FTC versus placebo at month 30 (p <0.05). The proportion of participants with BMD losses of >3.0% at any anatomic site was higher for FTC-TDF vs placebo (50.0% vs 32.9%, p =0.04); no benefits for calcium supplementation for participants with a low baseline BMD were observed. Of note, completion rates were very low in that sub-study (<70%).

HIV resistance

One concern commonly raised about the safety of TDF PrEP is the potential for selection of HIV resistance. Resistance can arise when PrEP is used by individuals with unidentified HIV infection (principally acute infection at the time of PrEP initiation) or in persons with inadequate adherence who experience breakthrough infection in the face of some drug pressure. Seven trials investigated cases of drug resistance to either FTC (the substitution mutations M184I/V) or TDF (K65R and K70E) PrEP[1-5,15,16,125-127], primarily using standard genotypic assays but with next generation ultrasensitive analyses in some cases. Overall, 9 out of 45 individuals with unrecognized acute HIV infection at randomization and assigned to either TDF or FTC-TDF had mutations that confer resistance to FTC or TDF, and so did 7 resistant HIV infections out of 271 cases of incident HIV infection post-randomization in individuals that were assigned either TDF alone or FTC-TDF[1-5,15,16,125-127]. The development of resistant mutations appeared to be related to FTC more commonly than TDF, consistent with a lower bar to selection of the

M184V mutation for FTC than the K65R mutation for TDF. Importantly, PrEP-related resistance faded to background levels after PrEP was stopped. These data suggest that selection for mutations that confer resistance is rare but occur primarily if PrEP is started with early HIV infection unrecognized by antibody testing done at the time of PrEP initiation. The population level effect of the PrEP-related resistance is still unclear but mathematical modeling suggest that the cumulative risk of drug resistance from PrEP could be much lower than that associated with that already known to be selected by antiretroviral treatment of persons with established HIV infection[128].

Sexual and reproductive health

Women at greatest risk for HIV are in their childbearing years exposed to both the risk for HIV infection and unintended pregnancy. PrEP and joint use of hormonal contraception could offer dual protection. Two studies reported detailed data on the effectiveness of hormonal contraception comparing participants randomized to receive active PrEP and those randomized to receive placebo. In the Partners PrEP Study, pregnancy rates and hormonal contraceptive effectiveness were statistically similar for women assigned PrEP versus placebo[129]. In the same cohort, the protective effectiveness for PrEP versus placebo was similar among women using DMPA and those using no hormonal contraception, 64.7% and 75.5% respectively (p=0.65)[130]. In the FEM-PrEP Study, pregnancy rates were not significantly different for women assigned FTC-TDF PrEP versus placebo after adjustment for age, contraception method and site [adjusted hazard ratios (95% confidence interval): 1.2 (0.9 to 1.8), p= 0.20][16,131]. These data suggest that TDF-based PrEP does not appear to affect the

effectiveness of hormonal contraception, nor does hormonal contraception affect PrEP efficacy.

Data on the safety for exposed fetuses are limited because in PrEP trials with heterosexual women medication was promptly discontinued for those who became pregnant, but some data are available from women who were on study drug in their first few months of pregnancy. In the Partners PrEP Study, among 288 women with first trimester (median= 35 days) TDF exposure[132], the frequency of pregnancy loss was 42.5% for women receiving FTC-TDF (p= 0.46) and 27.7% on TDF alone (p=0.16) vs 32.3% on placebo. In addition, there were no significant differences in pregnancy-related and infant adverse outcomes including preterm birth, congenital anomalies, and growth throughout the first year of life for infants born to women who received PrEP vs placebo. Similarly, pregnancy rates and related adverse outcomes were similar for women assigned FTC-TDF or TDF alone versus placebo in the VOICE Study[15]. In a Ugandan study of safety of daily/intermittent FTC-TDF PrEP versus daily/intermittent placebo[116], 3 pregnancies were recorded overall, resulting in one live birth (daily placebo group) and 2 losses: one spontaneous abortion at 6 weeks of pregnancy (daily active group) and a molar pregnancy (intermittent placebo group). Taken together, these data although limited by small numbers and follow-up duration suggest no clinically significant risks for poor birth outcomes or infant growth among women with early pregnancy TDF exposure and are consistent with evidence from HIV-infected women using tenofovir for treatment[133,134] and data on pregnancy and infant outcomes

in the US Antiretroviral Pregnancy Registry involving >3000 pregnancies with first trimester exposure to either FTC or TDF[135].

Another concern in the sexual and reproductive health space is the theoretical potential for increased sexual risk behaviors as a result of using PrEP, a concept referred to as risk compensation. Risk compensation could lead to a heightened risk of HIV acquisition, overwhelming PrEP's protective effects, or could expose individuals to other adverse effects (sexually transmitted infections, unintended pregnancy). However, data to date do not indicate substantive sexual behavior with PrEP. In PrEP clinical trials, risky behavior declined over time, including declines in the frequency of sex acts unprotected with condom, number of sexual partners, and sex with partners with unknown HIV status compared to baseline levels[1,4,16,116,117,136-140]. Importantly, PrEP use and efficacy appear to map best to individuals who are not using condoms already at the time they start PrEP.

Comparison with safety of potential alternative PrEP drugs

Currently FTC-TDF in the US is the only medication with a label indication as PrEP against HIV acquisition. However, new PrEP drugs and formulations are currently being evaluated including other oral agents (e.g., maraviroc), intravaginal rings (dapivirine, tenofovir), and longer-acting injectable agents (rilpivirine, cabotegravir). These agents appear to have good safety profile when used for treatment of HIV infection but their efficacy and clinical safety as PrEP in HIV-uninfected persons is still unknown.

Tenofovir alafenamide (TAF) a newly-developed prodrug for tenofovir that delivers 90% lower plasma tenofovir concentrations compared with standard TDF, has recently received US FDA approval for treatment of HIV infection (approval first as a coformulation with elvitegravir/cobicistat/emtricitabine). TAF, compared to TDF, appears to result in less potential for kidney and bone toxicity in HIV-infected persons. However, TAF, alone or in combination with emtricitabine, has not been formally evaluated as PrEP in HIV-uninfected populations, although its potential for offering a PrEP option with further-diminished long-term toxicity holds substantial appeal.

Conclusions

TDF-based PrEP is a recommended approach to prevention of HIV acquisition in combination with other HIV prevention strategies. It is highly effective against HIV acquisition when taken. A PrEP start-up syndrome with gastrointestinal symptoms is the most common side effect but symptoms are self-limited. TDF-based PrEP is associated with modest but statistically significant declines in both eGFR and BMD but the declines are non-progressive, and not associated with clinically relevant glomerular dysfunction and bone fractures, respectively, and quickly resolved after TDF discontinuation. HIV resistance selected by PrEP is rare but can occur if PrEP is initiated with unrecognized acute HIV infection and is mostly associated with mutations that confer resistance to the FTC component. Sexual and reproductive health concerns related to PrEP have not been borne out by current data. Of note, the low adherence to PrEP reported in some of the first generation PrEP trials may limit the strength of evidence from these study populations. Limitations of low drug exposure in some studies and short follow-up notwithstanding, these data suggest that oral TDF-based PrEP may be associated with

limited clinically relevant safety signals in healthy persons, but safety among subpopulations with co-morbid conditions like reduced kidney function, diabetes, hypertension or concurrent nephrotoxic medication is still unknown as does the long-term effects beyond reported study durations.

Expert opinion

Oral TDF-based PrEP is an effective and FDA approved novel strategy to reduce risk of HIV acquisition. Normative agencies have issued guidelines for use of PrEP in clinical settings as a prevention option to persons at substantial risk for HIV acquisition. Clinical experience has been accumulated from randomized clinical trials and observational studies involving more than 10,000 HIV-uninfected adults from diverse geographies and at-risk groups, exposed to TDF-based PrEP ranging from 6 months to 5 years. These data suggest that oral TDF-based PrEP is generally safe and well-tolerated in healthy HIV-uninfected populations. TDF-based PrEP carries a small but statistically significant risk for renal and bone toxicity, somewhat limited data for safe use in pregnancy and lactation, as well as potential to select for HIV resistance to PrEP medications, if PrEP is used by persons with established HIV infection. Renal and bone effects of TDF-based PrEP appear to resolve on drug discontinuation. The US CDC recommends monitoring of renal function at 3 months and bi-annually based on creatinine clearance after initiating PrEP but given that clinically relevant renal toxicities are rare and appear to resolve quickly after stopping TDF, less renal monitoring may be sufficient. Tubular renal toxicity from PrEP appears to be very rare and active screening is not recommended. Although the clinical significance of the observed bone loss on future risk for fragility fractures is not clear, current evidence does not support radiologic

assessments of bone health before the initiation of PrEP or for the monitoring of persons while taking PrEP. A new formulation of tenofovir, tenofovir alafenamide (TAF), has recently been developed and appears to demonstrate favorable renal and bone safety profile compared to TDF but efficacy and safety as PrEP is yet to be proven in HIV-uninfected populations. Regarding safety during pregnancy and breastfeeding, current data from first trimester exposure TDF PrEP and collaborated with data from the US Antiretroviral Pregnancy Registry (sufficient enough to rule up to 2 fold increase in risk of birth defects associated with TDF exposure) do not cause concern for PrEP use during early pregnancy, but effects of TDF-based PrEP used the entire pregnancy and early postpartum are essentially unknown. Selection for mutations that confer resistance to TDF and FTC is rare but risk exist primarily when PrEP is initiated with unrecognized HIV infection; mathematical modeling studies suggest that the cumulative risk of drug resistance from PrEP is expected to be low. In addition, PrEP breakthrough infections are rare and this risk must be weighed against the number of new HIV infections averted by PrEP. Importantly, the observation that resistant mutations faded to background level after stopping PrEP is reassuring suggesting that the risk of compromising subsequent combination treatment options for a minority of individuals with PrEP breakthrough infections might be low and of limited public health consequence. In summary, TDF-based PrEP is a highly potent HIV prevention strategy for which the observed risks arguably are outweighed, at the public health level and for at-risk individuals, by its HIV prevention benefits.

Table 0:1. Drug summary box

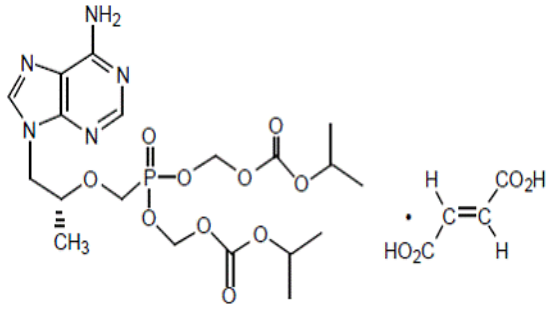
Drug name	Tenofovir Disoproxil Fumarate (TDF)
Phase	Initial US FDA approval for treatment of HIV infection: 2001 US FDA approval of emtricitabine (FTC)-TDF for prevention of HIV infection: 2012
Indication	Prevention and Treatment of HIV infection
Pharmacology description	An oral prodrug of tenofovir, an acyclic nucleotide (nucleoside monophosphate) analogue with activity against retroviruses, including HIV-1, HIV-2 and hepadnaviruses
Route of administration	Oral
Chemical structure	
Pivotal trials for Safety and Efficacy against HIV acquisition	1. The Partners PrEP trial was a randomized, double-blind, placebo-controlled 3 arm trial of daily TDF alone or in combination with FTC in 4747 serodiscordant heterosexual couples in Kenya and Uganda[1] 2. The iPrEx trial was a randomized double-blind placebo-controlled multinational study evaluating FTC-TDF in 2499 HIV-seronegative men or transgender women who have sex with men and with evidence of high risk behaviour for HIV infection.[3]

Table 0:2. Summary of clinical safety of oral tenofovir disoproxil fumarate-based pre-exposure prophylaxis for HIV prevention

Safety signal	*Reference contributing to evidence*	*Summary/range of effects for TDF-based PrEP vs placebo or no PrEP*	*Current recommendation*
PrEP start-up syndrome	1-3,6,7,15,16,115-119	Mild gastrointestinal symptoms (primarily nausea but also vomiting, diarrhea, abdominal pain) are the most common side effects associated with PrEP. Occurred 1-18% of participants assigned PrEP vs 0-10% of participant's assigned placebo in clinical trials but end spontaneously within limited 1-2 months.	Counsel clients on potential side effects
Kidney toxicity	57,61,62	1-5 mL/min/1.73m^2 net mean eGFR decline associated with PrEP vs placebo with no clinically relevant eGFR decline from baseline observed up to 5 years. Declines resolve with drug with discontinuation. Limited data on tubular dysfunction but no evidence of difference in prevalence for PrEP vs placebo seen in the iPrEx sub-study. **<u>Study specific net eGFR/CrCl decline attributable to PrEP</u>** **Partners PrEP Study (CKD-eGFR):** -1.23 to -1.59 mL/min/1.73m2 over 1.8 per-protocol months. Proportion of persons with ≥25% eGFR decline at 12 months: 1.3-1.8% for PrEP vs 1.3% for placebo. **iPrExStudy (CrCl):** -1.3 mL/min at 4 weeks and -1.5 mL/min -1.5 mL/min at last-on treatment visit , observed over 81 weeks (p=0.02). No differences in markers of proximal tubular dysfunction between PrEP and placebo. **Bangkok Tenovofir Study (CrCl):** -2.5 mL/min at 24 months and -5.2 mL/min at 60 months.	During PrEP use, current CDC guideline are: a) Initiate PrEP in person with CrCl ≥ 60mL/min, and no contraindicated medications. b) Monitor renal function at 3 month and then bi-annually using CrCl not serum creatinine alone
Bone toxicity	122-124	Overall, ≤1.6% net loss in BMD associated with PrEP vs placebo over 1-2 years. No elevation in risk for bone fractures has been reported. Decline resolves to baseline with TDF discontinuation. **<u>Study specific net BMD decline attributable to PrEP vs placebo/no PrEP</u>** **iPrEx Study (at week 24):** Hip: -0.61% (p=0.001); lumbar spine: -0.91% (p=0.001) **TDF2 Study (at month 30):** Forearm:-0.86% (p=0.008); Spine: -1.64% (p <0.001Hip: -1.55% (p=0.001); Proportion of persons with ≥3% bone loss at any site: 50% vs 32.9%; p=0.04. **CDC Safety Study**: Femoral neck: -1.1% (p=0.004); Total hip: -0.8% (p=0.003); Lumbar spine L2-L4: -0.7% (p=0.11); Proportion of persons with ≥5% bone loss at any site at 24 month: 13% vs 6% (p=0.1)	Laboratory or radiologic assessments of bone health is not recommended before the initiation of PrEP or for the monitoring of persons while taking PrEP
Antiretroviral Resistance	1-4,6,15,16,125-127	HIV mutations that confer resistance to FTC (M184I/V) or TDF (K65R and K70E) are rare but can occur, principally with unrecognized acute infection at the time of PrEP initiation. In all reviewed studies, resistant mutation were observed in 9/45 and 7/271 of persons with unrecognized acute HIV	Documented negative HIV test result before prescribing PrEP, and no signs/symptoms of

		infection at PrEP initiation and PrEP breakthrough HIV infection (i.e. persons who were HIV uninfected at the initiation of PrEP), respectively. Mostly affects FTC component than TDF and appeared to fade to background levels after PrEP was stopped	acute HIV infection. HIV test every 3 months.
PrEP-hormonal contraception interaction	129-131	No evidence that PrEP affects hormonal contraception effectiveness and vice versa	PrEP can be used among women using contraception for pregnancy prevention.
Pregnancy adverse events	116,131,132	Limited data from first trimester TDF exposure suggest no increased risk for poor birth outcomes and no delays in infant growth during the first year of life. Eg. Pregnancy loss in Partners PrEP study (n=288 pregnancies): 42.5% for FTC-TDF (p=0.16) and 27.7% for TDF group (p=0.46) vs 32.3% placebo	US FDA labeling information permit PrEP use for preconception and during pregnancy by the uninfected partner as it may offer an additional tool to reduce the risk of sexual HIV acquisition, recognizing that the amount of data is limited. Assess pregnancy intent while on PrEP and counsel based on available data.
Behavior risk compensation	1-6,15,16,116,117,136-140	Evidence of substantial behavior risk compensation is limited. Current data generally show declines in sex acts unprotected with condom, number of sex partners, having sex with partners of unknown status during follow-up and no difference in objective indicators of unprotected sex like incident STI infections and pregnancy compared to placebo or no PrEP (including baseline)	Consider the epidemiologic context of the sexual practices reported by the individual. Provide behavioral risk reduction support, medication adherence counseling, and STI symptom assessment at start and while on PrEP.

BMD: bone mineral density; CrCl: creatinine clearance; eGFR: estimated glomerular filtration rate; FTC: emtricitabine; PrEP: pre-exposure prophylaxis; STI: sexually transmitted infection; TDF: tenofovir disoproxil fumarate.

Conclusion

Conclusions

Daily oral TDF-based PrEP is a recommended and highly effective approach for prevention against HIV acquisition when taken with sufficient adherence. For any biomedical prevention intervention including pharmacological interventions like PrEP, providing a daily medication to healthy, HIV-uninfected persons requires an extraordinarily high degree of safety since the person is taking the medication to prevent an infection that might not occur. In addition to the potential deleterious clinical effects, safety concerns can have a big impact on the intervention uptake and effectiveness if people choose to skip doses or stop their drugs altogether. We have provided a comprehensive investigation as well as a review of all potential off-target effects of TDF-based PrEP for HIV prevention. These data are both novel and provide important empirical evidence base to support safety of TDF based PrEP as part of a comprehensive HIV prevention package. These data may not only accelerate PrEP implementation but will also enhance PrEP adherence for effective pharmacological chemoprophylaxis for at-risk individuals using PrEP.

Interpretation of Findings:

Chapter 2: Effect of TDF-based PrEP on eGFR in HIV-uninfected men and women. An important concern for the use of TDF for PrEP is the potential for kidney toxicity including decline in eGFR. In chapter 2, we investigated whether TDF-based PrEP causes clinically relevant decline in eGFR. Our data shows that daily oral TDF-based PrEP was associated with a small but statistically significant decrease in eGFR – specifically, a change relative to baseline <1.5%, which was non-progressive for 36 months and was not accompanied by a significant increase in the likelihood of a

clinically-relevant change in eGFR (i.e., ≥25%). Importantly, the observed declines in eGFR resolved within weeks of TDF discontinuation including in a minority of participant who experienced clinically relevant eGFR decline[141] (Figure 0:1).

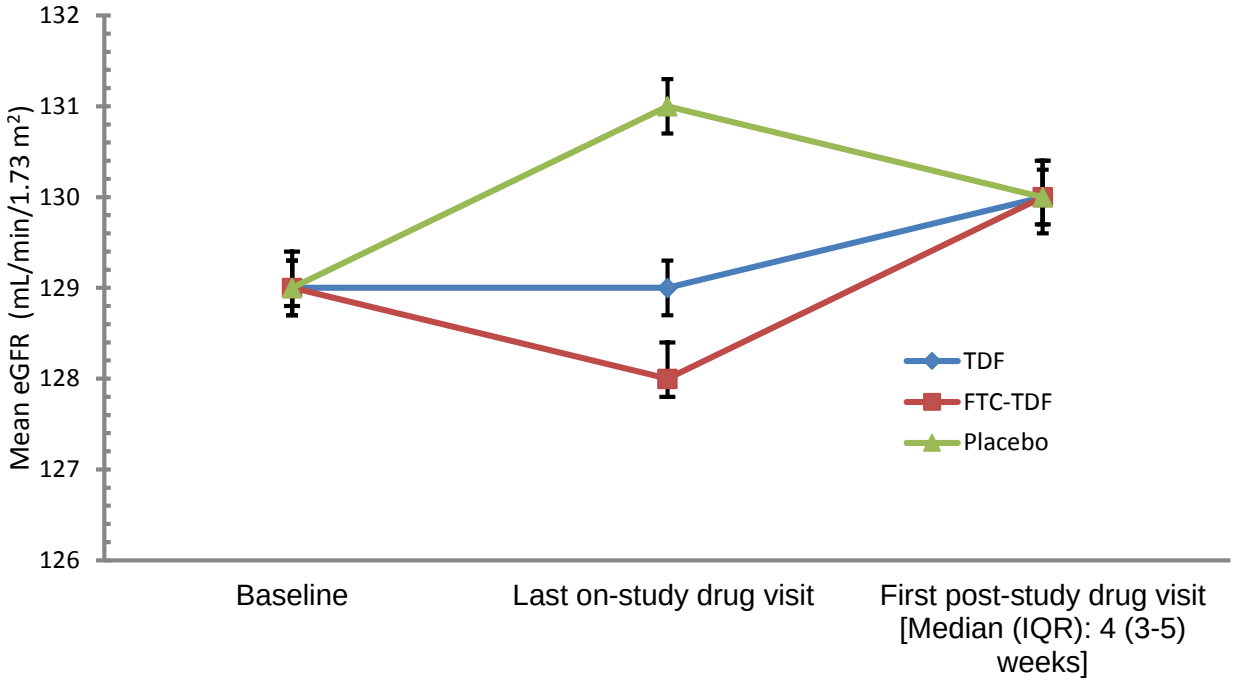


Figure 0:1. Mean eGFR at the last on-study drug visit and the first post-study visit after discontinuation of study drug, according to treatment group.
(Adapted from Mugwanya et al JAIDS 2016)[141].

Because PrEP use is a time-dependent intervention for months or years of greatest HIV risk and not life-long, the clinical significance of the observed changes in eGFR may be quite small. In the studied cohort, plasma drug levels were available from a random sample of 200 persons. Overall, tenofovir concentrations were detected in 82% of the subjects;[56] concentrations with threshold of >40 ng/mL –a level that is consistently achieved with daily dosing but are also likely in persons who took a single dose in the

last 24 hours– were consistently high through 36 months after randomization. Thus, these data provide consistent evidence of high PrEP exposure in this study population, lending great importance to our final findings.

Chapter 3: Effect of TDF-based PrEP on proximal tubular dysfunction in HIV-uninfected men and women. The primary consequence of TDF exposure is proximal tubular dysfunction, which can occur without severe decline in the GFR. In chapter 3, we showed that proximal tubular dysfunction was rare (including Fanconi syndrome) among >1500 HIV-uninfected men and women with high adherence to daily oral FTC-TDF PrEP up to 24 months of observation. Higher frequency of isolated tubular proteinuria and isolated hyperuricosuria occurred commonly in persons on FTC-TDF compared to placebo. Because total proteinuria and albuminuria have been associated with increased risk of adverse outcomes, further research is needed to determine the clinical significance of non-albumin proteinuria. These findings suggest that monitoring with routine urine markers of proximal dysfunction will not be an efficient approach to predict this rare but serious adverse renal event with PrEP. These findings, together with complementary findings of an optional substudy of iPrEx study, which demonstrated a very low rate of proximal tubulopathy in men predominantly enrolled in South America, suggest that monitoring with routine urine markers of proximal tubulopathy will not be an efficient approach to predict this rare but serious adverse renal event with PrEP. Taken together with our findings in Chapter 2 addressing the effect TDF PrEP on eGFR, our data provides robust evidence suggesting that clinically relevant kidney toxicities are

rare in HIV-uninfected persons using TDF-based PrEP and readily resolve within weeks of PrEP discontinuation.

Chapter 4: Infant exposure to FTC-TDF PrEP via breastmilk: The standard for use of drugs during pregnancy and lactation is very high due to safety concerns for unborn or breastfeeding infant. As PrEP becomes widely used in heterosexual populations,an important consideration is its safety in infants who are breastfed by women taking PrEP for HIV prevention. In a prospective, pharmacokinetics study of infant exposure to FTC-TDF PrEP via breastmilk, we found that infants had low exposures to tenofovir and emtricitabine, which would not be expected to pose substantial safety risk to infants of mothers who use PrEP during breastfeeding. Specifically, the estimated infant doses from breast milk and resultant infant plasma concentrations for tenofovir and emtricitabine were 12,500 and >200-fold lower than the 6-mg/kg that has been proposed for infant therapeutic doses and tenofovir was not detected in 94% of infant plasma samples. These data are a first for the field and are informative for evidence-based clinical practice guidelines. Our findings do not only allay the fear about the consequences of infant exposure from maternal PrEP medication but will also permit effective pharmacologic chemoprophylaxis for at-risk women who desire to conceive or breastfeed while still able to reduce their HIV risk using PrEP. For breastfeeding women taking oral TDF PrEP, breast milk exclusively contains tenofovir in an unconjugated anionic form, and due to its poor oral bioavailability, negligible tenofovir concentrations would be expected to be absorbed by the infant from breastfeeding, consistent with our

findings. As expected and consistent with the structurally similar lamivudine, emtricitabine which is readily bioavailable after oral ingestion, was excreted into breast milk and was quantifiable in the infant plasma, but the concentration we observed in this study was a small fraction (~0.5%) of doses proposed in infant prophylactic daily doses to prevent vertical HIV acquisition. The effects of chronic infant exposure to low concentrations of PrEP drugs including the potential resistance if the mother using PrEP gets infected with HIV are essentially unknown, but given the amount of breast milk an infant consumes daily, estimated to range from daily mean intake of 574 ± 60 mL to 1181 ± 94 mL over the first 6 months,[142] these concentrations are likely to be of limited clinical consequence. The presence of some emtricitabine concentration in infant plasma raises an important question on the possibility of viral resistance to emtricitabine in infants breast-fed by mothers using FTC-TDF PrEP in the event of maternal HIV infection. However, this risk is only downstream conditioned on maternal HIV acquisition first but for mothers taking PrEP, the risk for getting HIV is very low risk. Moreover, for a mother with PrEP break-through HIV infection, the immediate concern would be about her selecting for and then transmitting resistant virus and it is also possible that the concentration of FTC in infant plasma may be too low to put selective pressure on the virus to select for resistance. After pharmacokinetic demonstration of minimal tenofovir and emtricitabine in infants breast-fed by mothers the next step should be exploration in implementation science studies the acceptability, adherence, and infant safety in women who choose to continue PrEP throughout their pregnancy or breastfeeding and the long-term effect of these low concentration exposure.

Chapter 5: Sexual behavior risk compensation. Open label extension of blinded randomized trials offer an opportunity to address important implementation questions that cannot adequately be addressed in blinded trial phase including sexual risk compensation. The transition from a blinded, placebo-controlled phase in the Partners PrEP Study among HIV serodiscordant heterosexual couples to an open-label extension in which all participants aware they were receiving active, efficacious PrEP provided a "natural experiment" to evaluate potential sexual risk compensation. We found no increase in unprotected sex in serodiscordant couples, STIs, or pregnancy, either immediately or over time before and after unblinding. Our findings provide encouraging evidence that behavioral changes as a result of PrEP might not undermine its strong HIV prevention and public health benefits. Of note, unprotected sex within the known HIV serodiscordant primary partnerships declined during follow up compared to baseline, suggesting that PrEP may be synergistic with other HIV prevention strategies including risk reduction counseling and condoms. Similar findings have since been reported in studies among men. Importantly, this study provides critical empirical data to inform culturally tailored behavioral counselling and assessment of strategies to minimize risk-taking and to maximize adherence. Furthermore, these data have usefulness in understanding bio-behavioral issues integral to PrEP roll-out. We observed a small increase in sexual acts with outside partners, but this was not accompanied with increase in clinical endpoints indicative of unprotected sexual activity (i.e. STIs and pregnancy rates were similar in both study periods). Although the

increased sexual activity outside the primary study partnership my suggest some form of risk compensation, our previous work showed that sex outside the partnership was mostly due to dissolved and reformed relationships.[99] Similarly, in the current study the proportion of individuals reporting sexual activity during follow-up with different partner types (Table 8:1) was consistent with our prior data – specifically, partnerships appear to dissolve over time at a modest rate, with some degree of new partnership formation. The decision to initiate an outside partnership is likely multifactorial and the modest increase in risk-taking behavior we observed is difficult to ascribe to a specific source. How to consider PrEP's utility for individuals previously in known HIV serodiscordant partnerships but now in new partnerships (and thus essentially like any other new partnership) is a topic that needs exploration.

Longitudinal studies are often threatened by alternative hypotheses including, for our study, the potential that over time higher risk-taking individuals would be more likely to contract HIV and, therefore, censored out depleting the analyzed cohort of high risk takers over time. However, by the time of unblinding (i.e. July, 2011), only 38 of 3163 (1.2%) HIV-uninfected participants randomized to the trial's active PrEP arms (and thus the focus of the presented analysis) had acquired HIV. Given this small number, it

Table 0:1. Proportion of individuals reporting sexual activity with different partner types

	WOMEN				MEN			
	Neither	**Primary partner only**	**Outside only**	**Both**	**Neither**	**Primary partner only**	**Outside only**	**Both**
Enrollment	2.5%	97.0%	0.0%	0.4%	3.8%	82.7%	0.5%	13.0%
Month 12	15.1%	83.2%	1.4%	0.3%	13.1%	68.1%	5.8%	13.0%
Month 24	19.8%	77.6%	2.1%	0.5%	15.3%	65.0%	9.1%	10.7%

seems unlikely that the overall risk behaviors in the analysis after unblinding were driven by these individuals. More relevant are secular trends in sexual behavior over time in this cohort –specifically, a decline in condomless sex acts within the known HIV serodiscordant primary partnerships, as a result of ongoing counseling, relationship dissolution, and other factors, and an increase in sexual activity with additional partners; those factors were accommodated by the longitudinal models implement for the analysis. During the Partners PrEP Study, subjects were informed of results of other PrEP trials as they became available. At the time the primary results of the Partners PrEP Study were made public, the iPrEx Study[3] (showing efficacy of PrEP among men who have sex with men, largely from the Americas, a very different population than the Partners PrEP Study population) and the FEM-PrEP Study[16] (failing to demonstrate efficacy among higher-risk African women) were available. Although it is possible that knowledge of those trial results may have influenced participants' sexual behavior, in effect hedging against potential non-efficacy of PrEP given conflicting trial results, our experience is that subjects' greatest focus was on the results of the trial for which they were participating. The Partners PrEP Study received substantial international and local media attention as well. Finally, as subsequent results were available (e.g., the VOICE study, which did not demonstrate PrEP efficacy among African women, largely from southern Africa),[15] those results were reported to participants, but it was emphasized that PrEP was protective against HIV acquisition in the Partners PrEP Study.

Chapter 6: Review of empirical literature on safety of TDF-based PrEP for HIV prevention. *A* high level of confidence of safety is needed for prevention interventions including PrEP, since the person is healthy and is taking the medication to prevent an infection that might not occur. In chapter 5, we provided a comprehensive review, synthesis and interpretation of current data on the safety of oral TDF-based PrEP in the context of PrEP's overwhelming protective effectiveness against HIV acquisition for HIV-uninfected adults with focus on TDF-related effects on tolerability, kidney function, bone density, HIV resistance, sexual and reproductive health.

Tolerability: In general, gastrointestinal symptoms (mostly nausea vomiting and diarrhea) are the most common side effect occurring 1-2 weeks of starting PrEP but symptoms are self-limited without requiring PrEP discontinuation.

Nephrotoxicity: Nephrotoxicity, an expected complication based on experience among HIV-infected populations, is rare when assessed both as glomerular or tubular dysfunction. Overall, modest (1-3 mL/minute) but statistically significant declines in eGFR occur with TDF-based PrEP use but the declines are non-progressive, and not associated with clinically relevant glomerular dysfunction. Proximal tubular dysfunction, including Fanconi syndrome has not been although isolated proteinuria has been in seen to occur more frequently in TDF study groups compared to placebo.

Bone toxicity: Modest (0.4% to 1.6%) but statistically significant net decreases in bone mineral density have been observed with TDF PrEP use among HIV-uninfected individuals but without elevation in the risk of bone fractures. However, studies reporting on bone mineral density were limited by low adherence to PrEP.

HIV viral resistance: Selection for mutations that confer resistance to TDF and FTC is rare but risk exist principally when PrEP is initiated with unrecognized HIV infection; mathematical modeling studies suggest that the cumulative risk of drug resistance from PrEP is expected to be low. Importantly, resistant mutations appear to disappear after stopping PrEP suggesting that the risk of compromising subsequent combination treatment options for a minority of individuals with PrEP breakthrough infections might be low and of limited public health consequence.

Reproductive health adverse outcomes: There is no evidence that PrEP affects hormonal contraception effectiveness and vice versa. Limited data available from first trimester TDF exposure suggest no safety concerns with no increased risk for poor birth outcomes and no delays in infant growth during the first year of life. Consistent evidence has been reported from HIV-infected women using TDF for treatment and data on pregnancy and infant outcomes in the US Antiretroviral Pregnancy Registry involving >3000 pregnancies with first trimester exposure to either FTC or TDF. Ongoing demonstration studies in HIV-uninfected women using tenofovir-based PrEP during the entire pregnancy will contribute important additional data on birth and infant safety outcomes.

Sexual risk compensation: As reported in Chapter 4, sexual risk compensation have not been borne out by current data; data generally show declines in sex acts unprotected with condom, number of sex partners, having sex with partners of unknown status during follow-up and no difference in objective indicators of unprotected sex like incident STI infections and pregnancy compared to placebo or no PrEP (including baseline). Importantly, PrEP use and efficacy appear to map best to individuals who are not using condoms already at the time they start PrEP.

Alternative PrEP drugs: Currently FTC-TDF is the only medication with a label indication as PrEP against HIV acquisition and is likely to remain so for a number of years. However, new PrEP drugs and formulations are under evaluation to broaden the range of PrEP options including other oral agents (e.g., maraviroc), intravaginal rings (dapivirine, tenofovir), and longer-acting injectable agents (rilpivirine, cabotegravir). These agents in current PrEP pipeline appear to have good safety profile when used for treatment of HIV infection but their efficacy and clinical safety as PrEP in HIV-uninfected persons is still unknown. Tenofovir alafenamide (TAF) a newly-developed prodrug for tenofovir, appears also to result in less potential for kidney and bone toxicity in HIV-infected persons on TAF compared to TDF. However, TAF, alone or in combination with emtricitabine, has not been formally evaluated as PrEP in HIV-uninfected populations, although its potential for offering a PrEP option with further-diminished long-term toxicity holds substantial appeal. A robust pipeline based on new drugs will not only improve

safety profile but can build upon current successes by advancing products that are easily deliverable and offer practical options that fit into the context of people's lives. The recent demonstration that a dapivirine vaginal ring offers protection against HIV is a step in the right direction.

Risk mitigation:

Like all pharmaceutical interventions, PrEP implementation framework is multifaceted including among others mechanism to minimize the risk for the rare but potential off-target effects. For PrEP, risk mitigation strategies include but limited to HIV testing, behavioral support to minimize risk compensation and maximize adherence, safety screening and monitoring. Normative organizations including the US CDC and WHO have issued guidelines for the delivery of PrEP in clinical settings, and guidelines for other settings have been developed or are in development. Also, as part of requirement for the FDA, FTC-TDF for PrEP they required a risk evaluation and mitigation strategy from Gilead Sciences to ensure safe and appropriate use of FTC-TDF PrEP. An abridged list of risk mitigation strategies adapted from the US CDC clinical guidelines is showed in **Table 0:2**. These strategies are not only important to safe use PrEP but they provide opportunities for post-licensure surveillance for rare events as well as answering pertinent implementation challenges of delivering a pharmaceutical prevention intervention. For instance regarding renal monitoring, the current US CDC guidelines recommend renal monitoring at 3 months after starting PrEP and semi-annually thereafter. Our data aggregated over several safety analyses might suggest

that renal monitoring using creatinine clearance for oral TDF-based PrEP could potentially be less frequent than in the CDC guidelines, unless there are comorbidities. As PrEP delivery moves in more real world settings, important implementation questions related to the optimal frequency of safety monitoring remain unanswered. Operation questions like; 1) Can serum creatinine safely be used instead of a creatinine clearance for assessing PrEP eligibility and/or continuation? 2) Can PrEP be initiated without a creatinine or hepatitis B test? 3) Is an annual creatinine/clearance testing sufficient for kidney safety monitoring? etc. These question are especially important for PrEP implementation in resources limited setting and should and many and many others formally be tested in implementation science studies with PrEP delivery.

In summary, clinical experience accumulated from diverse populations show that oral TDF-based PrEP is generally safe and well-tolerated in HIV-uninfected populations, for which the observed risks arguably are outweighed, at the public health level and for at-risk individuals, by its powerful HIV prevention benefits. These data support the use of PrEP in combination with other HIV prevention strategies to reduce HIV acquisition risk.

Table 0:2. Risk mitigation strategies

Potential off-target effects	Recommended approach to minimize risk
Start-up syndrome	• Counsel clients on potential side effects
Sexual risk compensation	• Adherence counseling and support • Behavioral risk reduction support • Test for bacterial STIs every 6 months
Kidney toxicity	• Initiate PrEP only in persons with normal renal function defined as creatinine clearance or eGFR ≥ 60mL/min • Monitor renal function at least every 6 months
Bone toxicity	• Laboratory or radiologic assessments of bone health not currently recommended before initiation of PrEP or for the monitoring of persons while taking PrEP
HIV viral resistance	• Document negative HIV test result before initiating PrEP • Confirm no signs/symptoms of acute HIV infection. • HIV testing at least every 3 months.
Pregnancy and birth outcomes	• Assess pregnancy intention every 3 months while on PrEP and counsel based on available data.
Hepatitis B infection flare	• Document hepatitis B virus infection • and vaccination status

Biography

Kenneth Mugwanya is a Physician by training. He obtained his medical training from Makerere University Medical School in Kampala, Uganda, and MS in Epidemiology and Biostatistics from Case Western Reserve University, Cleveland Ohio. His research interests focus on risk factors for HIV and other STIs, Reproductive health, pharmacoepidemiology, and implementation science for novel prevention interventions.

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