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Evaluating Factors Associated with Tenofovir-Related Kidney Injury in HIV-Infected Women

By

Sanjiv M. Baxi

A dissertation submitted in partial satisfaction of the
requirements for the degree of

Doctor of Philosophy
in
Epidemiology
in the
Graduate Division
of the
University of California, Berkeley

Committee in charge:
Professor Arthur L. Reingold, MD, Chair
Professor George W. Rutherford, MD, AM
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Abstract

Evaluating Factors Associated with Tenofovir-Related Kidney Injury in HIV-Infected Women

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Doctor of Philosophy in Epidemiology

University of California, Berkeley

Professor Arthur L. Reingold, MD, Chair

Antiretroviral drugs are used in the treatment of human immunodeficiency virus (HIV) infection, and the global development and availability of such medications has expanded dramatically over the last 15 years. Currently, HIV-infected individuals require lifelong antiretroviral therapy, but if taken as prescribed and relatively early in the course of infection, persons living with HIV can expect to have a virtually normal lifespan. Although antiretroviral drugs are generally well tolerated, some side effects from treatment can be irreversible. Side effects are particularly important in the current HIV treatment paradigm, where individuals will require lifelong antiretroviral therapy. Tenofovir, currently formulated as tenofovir disoproxil fumarate (TDF), is a nucleotide analogue reverse transcriptase inhibitor that is one of the most commonly used medications in the treatment and prevention of HIV infection. Tenofovir has few known side effects overall, but long-term use has been associated with the development of a chronic, irreversible decline in kidney function in a small proportion of individuals. Other than cumulative duration of exposure to tenofovir, little else is known about what factors increase an individual's risk of developing kidney disease in the setting of chronic tenofovir use. As treatment for HIV infection has expanded in the developing world, with millions of people globally now taking tenofovir-containing products, understanding the factors that lead to a decline in kidney function in such individuals is fundamental to ensuring effective, yet safe, treatment and prevention of HIV.

Through this dissertation, I conducted four studies that attempted to improve the understanding of the factors that contribute to tenofovir-associated kidney damage. To test the assumption that the magnitude of exposure to tenofovir (that is, the amount of drug experienced by an individual with a given dose) likely affects an individual's risk of toxicity, an assumption that is frequently validated in pharmacodynamic studies of pharmacologic agents, I conducted three related studies. All three studies used a highly reliable measure of drug exposure – after taking a witnessed dose of tenofovir, study participants underwent serial blood sampling over 24 hours to draw a comprehensive exposure curve over a single day, yielding an area under the time-concentration curve (AUC) measure. The first study investigated the factors that contribute to elevated tenofovir exposure as measured by AUC. Interestingly, lower body weight, older age, pre-existing chronic kidney disease and

simultaneous use of a protease-inhibitor antiretroviral called ritonavir, were all associated with large increases in tenofovir AUC. Taking this information, in the second study, I investigated whether exposure to elevated levels of tenofovir were associated with a decline in kidney function over time. We found that increasing exposure to tenofovir at a fixed point in time is associated with subsequent decline in kidney function, but that this process is heterogeneous, with tremendous variability, particularly in the group with the highest AUC at baseline. In the third study, I attempted to identify whether the presence of distinct single nucleotide polymorphisms was associated with an elevated AUC. We found one polymorphism that was associated with large increases in AUC. This same polymorphism has been implicated in diseases related to transport of other organic anions, particularly the condition of uric acid metabolism called gout. This group of three studies established novel factors, both clinical and genetic, that are associated with an elevated tenofovir AUC and have helped to clarify whether AUC is a predictor of the ultimate development of kidney dysfunction. In a fourth study, as a bridge to a future research program, I investigated the predictors of urinary biomarkers of kidney injury in women living with HIV. We identified distinct patterns of novel urinary biomarkers as a function of the degree of control of HIV through treatment, but these findings will require clarification in a longitudinal cohort study. Given that intensive pharmacokinetic analyses are impractical in the clinical setting, this latter study will provide the basis for understanding what urinary biomarkers can help identify early evidence of a propensity to develop kidney injury in persons living with HIV. All of these studies took place in a cohort of predominantly black and Hispanic women, a group that is typically under-represented in both HIV and pharmacological research.

These four studies have improved our understanding of the factors that lead to the development of tenofovir-associated kidney disease in persons living with HIV. The findings have important implications for millions of people currently taking or planning to take tenofovir globally. As my career progresses, these studies have provided a framework that can be applied to studying drug toxicity in other settings or to delving more deeply into understanding the pharmacology of tenofovir and related compounds.

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PLEASE NOTE: The chapters included in this dissertation are not final manuscripts. All manuscripts will be published and openly accessible by the time that this dissertation is made public, so please refer to the finalized published manuscripts.

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Chapter 1: Common Clinical Conditions – Age, Low BMI, Ritonavir Use, Mild Renal Impairment - Affect Tenofovir Pharmacokinetics in a Large Cohort of HIV-Infected Women

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Chapter 2: Higher Tenofovir Exposure is Associated with Longitudinal Declines in Kidney Function in Women Living with HIV

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Chapter 3: Evaluating the Association of Single Nucleotide Polymorphisms with Tenofovir Exposure in a Diverse Prospective Cohort of Women Living with HIV

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Chapter 4: Changes in Urinary Biomarkers over 10 Years is Associated with Viral Suppression in a Prospective Cohort of Women Living with HIV

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MAIN TEXT

BACKGROUND

Treatment for human immunodeficiency virus (HIV) infection has seen dramatic changes in the last twenty years, but the current therapeutic paradigm still requires lifelong treatment with antiretroviral medications. In 2013, there were an estimated 29.2 million persons living with HIV globally and 1.8 million incident infections [1]. Since 2000, the number of individuals who are taking antiretroviral therapy for the treatment of HIV has increased dramatically, largely due to the concerted efforts of governments, governmental organizations and non-governmental organizations (see Appendix A, **Figure 1**). Currently, it is estimated that, globally, 15 million people are on antiretroviral therapy (ART), with a goal of 81% of all HIV-infected individuals being on antiretroviral therapy by 2020 [2, 3].

Tenofovir pharmacokinetics and change in kidney function. Since the U.S. Federal Drug Administration approved tenofovir disoproxil fumarate (TDF) in 2001, it has become one of the most frequently prescribed antiretroviral medications (ARV) used in the management of HIV infection [4]. Although formulated as the prodrug TDF to promote oral absorption and tissue delivery, tenofovir (TFV) or its' phosphorylated metabolites (TFV-DP, TFV-TP) are the active forms of the drug. TDF is a nucleotide analogue reverse transcriptase inhibitor that is co-formulated into several fixed-dose combinations, which help promote adherence to combination antiretroviral (cART) [5]. Furthermore, the co-formulation of TDF and emtricitabine is the only agent approved for pre-exposure prophylaxis of HIV infection in the United States [6, 7], where HIV-uninfected high-risk individuals take daily oral prophylaxis to prevent the acquisition of HIV. Lastly, tenofovir is also effective against hepatitis B virus (HBV) infection, which affects 240 million people worldwide [8]. Tenofovir is one of two medications recommended by the World Health Organization (WHO) for the treatment of those with chronic HBV infection [8]. Partly due to global non-governmental organization intervention, the price of tenofovir has been dramatically reduced in the regions of the world where HIV infection is most common, tipping the balance in favor of the cost-effectiveness for HIV treatment [9, 10]. The dramatic increases in ARV coverage seen globally [3] in recent years have largely been due to the availability of generic formulations of tenofovir-containing compounds [9, 10].

Tenofovir is considered by WHO to be an essential medication and a preferred first line agent in the treatment of HBV and the treatment and prevention of HIV [8, 11, 12]. While generally safe and effective, use of TDF has been associated with an increased risk of both acute and chronic kidney dysfunction [13], an effect that can trigger a discontinuation of the drug and a change in antiretroviral treatment regimen. The development of chronic kidney disease (CKD) in the setting of HIV infection is multifactorial [14], but irrespective of HIV infection, the development of CKD is associated with increased morbidity and mortality [15, 16]. There is sufficient evidence to conclude that TDF can lead to significant kidney toxicity in some individuals and that this toxicity may be a function of longitudinal exposure (defined in one study as number of visits on drug) [13, 17-21]. Moreover, TDF-associated renal toxicity may not be reversible, even with discontinuation of the drug [13, 18]. Therefore, understanding who is at risk for developing tenofovir-related kidney injury, and providing them with an alternative

antiretroviral regimen from the time of initiation of ART, is the ideal approach to addressing this potential complication of ARV treatment.

Adverse effects of medications are often correlated with systemic levels of the drug. As is common with many medications, TDF demonstrates significant inter-individual variability in plasma drug levels of TFV [22, 23] (see Appendix A, **Figure 2**). Once an individual takes a medication, the factors that govern his or her drug "exposure" are collectively referred to as pharmacokinetics (PK) and include the absorption, distribution, metabolism and excretion (ADME) of a given compound. Inter-individual variability in tenofovir exposure due to differences in pharmacokinetics has been hypothesized to be the primary determinant of kidney injury, but studies to date examining this relationship have been limited by small sample sizes and a short duration of observation; by their use of study populations with limited generalizability; and by the use of surrogate assessments of tenofovir pharmacokinetics that may inadequately measure drug exposure [24, 25]. The factors that contribute to inter-patient variability in TFV PK in diverse and real-world populations are, therefore, largely unknown.

As with many medications, the TDF dose that is marketed for use in adults (300 milligrams by mouth once daily) was determined in Phase I and II studies [26]. Dose-finding studies entail intensive PK evaluations of short-term use in a limited number of volunteers (frequently HIV-uninfected) who are often homogeneous with regard to race/ethnicity, sex, the presence or absence of comorbid medical conditions, and, in the case of tenofovir, having normal kidney function at baseline. The generalizability of the findings of these studies to diverse patients with different characteristics is thereby limited by small sample sizes and relatively restrictive inclusion criteria [27-29]. Due to the limitations in post-marketing tracking procedures and publication bias, discovery of important factors that modify a drug's pharmacokinetic profile with use in more diverse populations is often delayed [30]. Furthermore, prior pharmacokinetic studies of TDF have not examined the relationship between variation in TFV exposure and subsequent kidney function.

In practice, an important limitation to the study of TDF is the dearth of information about the impact of clinical and host genetic factors on TDF exposure. Very few studies have explored what factors determine TFV exposure outside the clinical trial setting or have evaluated the impact of genetic parameters on TFV concentration [31]. Those studies of the effect of host genetics have focused on excretion of TFV (the active drug) via the kidney, which is not the sole determinant of exposure [32-37]. Pharmacogenetics are those genetic factors that determine pharmacokinetics (i.e., ADME) of a given drug. A limitation of pharmacogenetic studies is that they often fail to consider non-genetic factors that can affect pharmacokinetics. In addition, such studies often rely on single measurements of drug levels that fail to comprehensively represent drug exposure. Identifying single nucleotide polymorphisms (SNP) that play a role in tenofovir pharmacokinetics will help clarify any contribution from individual genetic variability in order to predict who may be at risk of drug-related toxicity.

Urinary biomarkers of kidney injury. After understanding the pharmacokinetics of TDF, including the clinical and genetic factors that affect its pharmacokinetics, and how TFV exposure may effect change in kidney function over time, there was still a need to identify readily accessible, cost effective, minimally invasive and highly accurate predictors of kidney injury in those taking TDF. Therefore, a final component of this dissertation was to investigate urine biomarkers of

kidney injury that may serve this purpose. Several urine biomarkers for use as early evidence of kidney injury have been proposed, including those predictive of progressive kidney decline and of incident CKD; these biomarkers often reflect different aspects of pathophysiologic mechanisms of kidney injury. Specifically, strong associations with subsequent decline in kidney function have been observed with the urine albumin to creatinine ratio (ACR), alpha-1-microglobulin (α1m) levels and interleukin-18 (IL-18) levels [19, 38-40]. Increased ACR has been associated with a subsequent decline in kidney function in a variety of disease states, including HIV infection; when increased, it is indicative of injury to the kidney's glomeruli [19, 40]. The α1m protein is a free radical scavenger that, when present at elevated levels in urine, is indicative of damage to the proximal tubules of the kidney [38]. Finally, IL-18 is a pro-inflammatory cytokine involved in cell-mediated immunity that, when present at elevated levels in urine, is also indicative of proximal tubular injury [19, 40]. In multiple analyses, with extensive covariate adjustment for factors that affect both inflammation and kidney function, each of these markers has been associated with both subsequent decline in kidney function and an elevated risk of mortality [19, 38, 39, 41].

Prior research has demonstrated an association between these three urinary biomarkers and an increased risk of both incident CKD and premature mortality among women with HIV-infection [19, 38, 40, 41]. However, in general, a major step in understanding the clinical utility of a biomarker involves establishing whether or not serial measures of the biomarker can capture longitudinal changes in health. Biomarkers are of little clinical value if they fail to reliably predict prognosis and, instead, reflect variability within the assay and random fluctuations in measurements. Therefore, biomarker research in persons living with HIV infection needs to evaluate the long-term changes in novel biomarkers and understand whether they are associated with changes in the risk of important health outcomes, including immune function. Therefore, in the fourth study, I investigated how HIV treatment, and associated improvement in immune function, predicts changes in three important urine biomarkers, α1m, ACR and IL-18, in women living with HIV, over a ten year interval. The results were surprising in that ACR did not change substantially and α1m was counterintuitively higher, although IL-18 was lower, consistent with the hypothesis that improved HIV control results in lower markers of inflammation.

Understanding the possible mechanisms of kidney injury. Appendix A, **Figure 3**, shows a general directed acyclic graph (DAG) that describes the factors that are being considered in chapters one and three in this dissertation. One potentially important factor that may lead to variability in tenofovir AUC is the genetic makeup of an individual. It is entirely possible, and likely true, that there are SNPs that influence how tenofovir is metabolized and thereby affect the degree of exposure to the drug and its metabolites experienced by a given individual. As outlined in chapter 3 of the main text below, through a comprehensive literature search, we identified a number of candidate genes that may be important contributors to the pharmacokinetic variability with respect to tenofovir. TDF was formulated once it was realized that TFV, the active drug form, has poor oral bioavailability. Once ingested, TDF is then absorbed and hydrolyzed from its diester form into TFV, which is then rapidly taken up by cells and subsequently phosphorylated two or three times intracellularly [42]. Tenofovir enters many types of cells, including kidney tubule cells. The drug is secreted into the kidney's tubules via

human organic anion transporter 1 (hOAT1) and possibly other kidney proteins, such as the multi-drug resistance-associated proteins (MRPs) [21, 42, 43] (see Appendix A, **Figure 4**). It is hypothesized that these proteins may be involved in determining an individual's effective, in vivo exposure to tenofovir. Other proteins may be involved in the secretion of tenofovir as well [44], but data concerning the roles of other proteins are very limited, and the genetic mechanism that underlies the observed variability in tenofovir pharmacokinetics remains largely unknown. Furthermore, other factors, such as body mass index (BMI), co-administration of other drugs, such as ritonavir or cobicistat, age and baseline kidney function, all are likely to play a major role in an individual's exposure to tenofovir [32, 45-52]. One important consideration in the analyses that examine how clinical factors affect tenofovir exposure, will be accounting for the presence of diabetes mellitus and hypertension. Both of these comorbid medical conditions can act as potential confounders or mediators, depending on the exposures and outcomes that are being considered. For example, both diabetes mellitus and hypertension are potential mediators on the path from age to tenofovir AUC but are likely both confounders of the relationship between baseline kidney function and tenofovir AUC.

In Appendix A, **Figure 5**, I have included a DAG of the subsequent path from AUC to kidney damage that reflects the hypothesized mechanism in chapter two. Age, BMI, race/ethnicity and duration of tenofovir are all plausible potential confounders of the relationship between tenofovir AUC and kidney injury. Given a reasonable set of assumptions, the same could be said regarding ritonavir use, HIV viral load (which is a surrogate for adherence to ART), CD4 T-cell count and diagnoses of either diabetes mellitus or hypertension. An assumption that is essential to acknowledge about the proposed mechanism of exposure being a primary determinant of subsequent decline in kidney function is that there is no effect of kidney injury on tenofovir AUC. This is an important assumption, because we know that baseline kidney function does, most likely affect AUC, and therefore, it is plausible that as tenofovir leads to kidney injury, this same progression of kidney disease would then affect AUC. In the studies for this dissertation, we are not measuring AUC serially; it is assumed that whatever AUC value has been measured and is available for analysis is the *least* possible AUC. If this assumption is incorrect the results would be biased toward the null (i.e., we would underestimate the impact of AUC on change in subsequent decline in kidney function), but serial monitoring of AUC among large numbers of participants is impractical, both logistically and financially, and the estimates we obtained from our approach are likely to be conservative, underestimating the effect.

Finally, possibly the most valuable contribution of this work is its focus on women, who are severely under-represented in pharmacokinetic studies. The Women's Interagency HIV Study (WIHS) cohort, in which these studies was undertaken, is very diverse with respect to age, race, ethnicity and socioeconomic status and accurately reflects the population of women infected with HIV in the United States [53]. Conducting studies in a representative population makes the results externally valid and generalizable to the populations from which the cohort was sampled. Few pharmacokinetic studies of antiretroviral drugs have been undertaken exclusively in women, and differences in the both the pharmacodynamics and pharmacokinetics in women have been well-documented for some time [54, 55].

Chapter 1: Common Clinical Conditions – Age, Low BMI, Ritonavir Use, Mild Renal Impairment - Affect Tenofovir Pharmacokinetics in a Large Cohort of HIV-Infected Women

Aim 1: Understand the clinical factors contributing to variation in pharmacokinetics, as measured by 24-hour tenofovir area under the time-concentration curves (AUC), in persons living with HIV who are on tenofovir-based antiretroviral therapy.

Sanjiv M. Baxi, Ruth M. Greenblatt, Peter Bacchetti, Rebecca Scherzer, Howard Minkoff, Yong Huang, Kathryn Anastos, Mardge Cohen, Stephen J. Gange, Mary Young, Michael G. Shlipak and Monica Gandhi.

INTRODUCTION

Since approval by the Federal Drug Administration in 2001, tenofovir disoproxil fumarate (TDF) has become one of the most frequently prescribed antiretrovirals in the management of HIV infection [4]. Moreover, TDF is co-formulated into several fixed-dose combinations, which can help promote adherence to combination antiretroviral therapy (cART) [5], and the co-formulation of TDF and emtricitabine is the only agent approved for pre-exposure prophylaxis in the United States [6, 7]. While mostly effective and safe, TDF has been associated with several major adverse effects in terms of renal function [13] and bone mineral density loss [56, 57], both of which can trigger discontinuation of the drug. Adverse effects of medications are generally correlated with systemic levels of the drug and, as is common with many antiretrovirals, TDF demonstrates significant inter-individual variability in plasma drug levels [22, 23]. The factors that contribute to inter-patient variability in TDF pharmacokinetics in diverse and real-world populations, however, are largely unknown.

As with most medications, the dose of TDF that was ultimately marketed for adults (300 milligrams (mg) once daily) with normal renal function was determined during Phase I and II studies of the drug [26]. Dose-finding studies usually entail intensive pharmacokinetic (PK) evaluations after short-term use in a limited number of volunteers (either HIV-infected or non-infected) who are often homogeneous with regard to race/ethnicity, sex and/or the presence of comorbid conditions. The generalizability of these PK studies to patients with different characteristics is thereby limited by their inclusion criteria and small sample sizes [27-29]. Broad recognition of important factors that modify a drug's PK parameters once it is used in more diverse populations can be delayed due to limitations in post-marketing tracking procedures or publication bias [30]. To address some of these limitations, we conducted intensive PK studies of TDF in a sample of HIV-infected women in the setting of routine clinical use in order to identify factors associated with drug exposure.

METHODS

Study population. The Women's Interagency HIV Study (WIHS) is a large, multicenter, prospective cohort study of HIV-infected women and at-risk HIV uninfected women in the United States [53]. The WIHS is highly representative of U.S. women living with HIV in terms of age, race/ethnicity, socioeconomic status, concomitant medications, comorbid medical conditions, etc. The "WIHS Intensive PK Study," has been described previously [58, 59] and enrolled a total of 480 HIV-infected women on different cART regimens for 12 to 24 hour

sampling of antiretroviral plasma levels after administration of a dose witnessed by study team members under conditions of routine participant use. For this analysis, our study sample consisted of WIHS participants who used TDF for at least six months prior to PK evaluation and underwent 24 hour intensive PK sampling (n=101 out of 117 met all criteria). See Appendix A, **Figure 6**, for a breakdown of the patients within the WIHS and those who underwent TFV PK evaluation. Institutional review boards at all participating institutions approved the consent and protocol materials for this study.

Intensive PK protocol methods. PK protocols were conducted in clinical research centers or other facilities associated with collaborating WIHS sites. Plasma samples were drawn over 24 hours for drug levels under conditions of actual use (including simulation of the participants' customary diet and administration of other medications). Participants were seen for the PK visit within six weeks of their core WIHS visit, and data were collected at both visits on weight, comorbidities, HIV RNA level, CD4 cell counts, self-reported adherence, hepatic and renal function and illicit substance use. All participants received standard dosing of TDF (300 mg orally once daily) and drug levels were measured in specimens collected at 0, 4, 8, 15, 18 and 24 hours after a witnessed dose.

Laboratory procedures. Plasma levels of TFV were determined by liquid chromatography/tandem mass spectrometry (LC-MS/MS) with TDF-d6 as the internal standard [60]. The plasma sample was pretreated with trifluoroacetic acid for protein precipitation before injecting into the Micromass Quattro Ultima LC-MS/MS system (Waters, Milford, MA). The assay was validated from 10 to 1000 nanogram (ng)/milliliter (mL) of TDF with a coefficient of variation < 15% for quality control samples at low, medium and high concentrations. Cystatin C was measured in 67 of the 101 women who contributed data to these analyses. Cystatin C in plasma samples was quantified as described previously [19] at the University of California, Los Angeles (UCLA) Clinical Immunology Research Laboratory by a particle-enhanced immunoturbidimetric assay (Gentian, Moss, Norway), which has been calibrated against the new World Standard Reference material ERM-DA471/IFCC [61]. Intra-assay coefficients of variation, based on 10 replicates, were <2% at serum concentrations of 0.7 and 1.1 mg/Liter (L). Inter-assay coefficients of variation were 4.4% and 3.9% at serum concentrations of 0.8 and 2.2 mg/L, respectively.

Predictor variables. We analyzed the following variables in relation to exposure: race (self-reported), age, concomitant medications (including ritonavir), HIV RNA, current and nadir CD4 counts, concurrent symptoms or infections, concomitant diabetes or hypertension, use of crack, powder cocaine, alcohol or tobacco, body mass index, estimated lean body mass, percent fat consumption in the diet via self-report and renal function parameters. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation using serum creatinine from the recent WIHS visit was employed to estimate glomerular filtration rate measures (eGFRcr) [62] in one set of models. EGFR was dichotomized as being more or less than 70 mL/minute (min)/1.73 meters $(m)^2$ in our models since this eGFR cut-off has clinical significance and the number of women with eGFR < 60 ml/min/1.73m^2 were few (n =3). The models were then repeated using the CKD-EPI equation for cystatin C (eGFRcys) [61-63]. Renal function prior to the initiation of TDF for each participant was also assessed using creatinine measures from prior visits (going back up to 4 visits before starting TDF if there were missing data). To obtain pre-TDF cystatin C for as many women as possible, we used values more than 4 visits back when necessary (n=16

of 67) from archived plasma specimens. All demographic data were collected at the core WIHS visit.

Outcome variable. Areas under the concentration-time curves (AUC) were used to estimate TFV exposure over the dosing interval; these were calculated for each individual using the trapezoidal rule [64]. Ten TFV concentrations at the beginning of the dosing interval (C_{mins}) in ten individual participants and one at the third sampling point were below the lower limit of quantification (10 ng/ml); all 11 observations were replaced by 0 ng/ml.

Statistical analysis. All analyses were conducted using Stata (version 11.2, College Station, TX) and SAS (version 9.2, SAS Institute, Cary, NC). For multivariate modeling, AUC was logarithmically transformed, and predictors' coefficients were back-transformed to produce estimated multiplicative effects on geometric mean AUCs. The multivariable model was constructed by forward stepwise selection, with $p<0.05$ required for entry and retention, but with race (African American versus others) included because of high *a priori* interest. Because 33% of the participants did not have available cystatin C measures from visits that preceded the start of TDF, we used multiple imputation [65] to reduce the likelihood of possible bias from excluding so many observations from analysis. Multiple imputation with the Markov chain Monte Carlo method was used to impute missing eGFR estimates using cystatin C, with ten imputations performed to yield ~95% relative efficiency [66].

RESULTS

Characteristics of patient population. TFV levels were measured over a 24-hour period for the WIHS participants. Appendix B, **Table 1** shows the patient characteristics of the study sample (n=101) and the distribution of relevant covariates, including those that remained in the final multivariate models. The mean age (range) of the participants was 43.1 (21.7-64.9) years. Sixty-four women (63%) reported their race as African American, 24 (24%) Hispanic and 10 (10%) Caucasian.

Summary of pharmacokinetic parameters for tenofovir. The TFV PK parameters for the study population demonstrated significant inter-individual variation. The median (range) for the PK parameters was as follows: TFV AUC 3350 (1031 – 13,911) ng x hours (h)/mL; minimum plasma drug concentration (C_{min}) 69.7 (0-363) ng/mL; maximum plasma drug concentration (C_{max}) 251 (81.1-1020) ng/mL; time after administration at which C_{max} was reached (t_{max}) 4.1 (0-24) hours; TFV clearance from plasma (CL/F) 322 (77-1047) mL/h. These data are summarized in Appendix B, **Table 2**, and Appendix A, **Figure 2** shows the time-concentration curves for the 101 participants who underwent intensive PK sampling for TFV levels. All PK curves and t_{max} values are included to reflect conditions of actual use in this cohort.

Factors associated with tenofovir AUC using eGFRcr. In the final multivariate model using the creatinine-based estimate of GFR, race did not substantially influence TFV exposure (Appendix B, **Table 3**), although older age was associated with higher exposure (increase in AUC by 1.21-fold for every decade of age, p=0.0007). Concomitant ritonavir (RTV) use (present in 61% of all participants) was associated with increased TFV AUC by an average of 1.33-fold (p=0.0020). Each 10% increase in body mass index (BMI, kilogram (kg)/m^2) was associated with a 0.96-fold reduction in TFV AUC (p=0.019). An eGFRcr of <70 mL/min/1.73m^2 prior to initiation of TDF was associated with a 1.31-fold higher AUC (p-value showed a trend towards statistical significance at 0.094).

Factors associated with tenofovir AUC using eGFRcys. In an alternative multivariate analysis (Appendix B, **Table 4**), eGFR was estimated using cystatin C measures and eGFRcys was made into a dichotomous variable as being ≥ or < than 70 mL/min/1.73m^2. As with the models using eGFRcr, race did not substantially affect exposure in this model (p=0.97). The effect of age on TFV exposure (1.20 fold increase in TFV AUC per decade of age, p=0.0003) was still prominent. Concomitant RTV use similarly increased exposure by an average of 1.33-fold (p=0.0014), and higher BMI was similarly associated with a lower (0.96-fold per 10% increase in BMI) TFV AUC (p=0.025). Mild renal insufficiency (eGFRcys of <70 mL/min/1.73m^2) preceding the initiation of the TDF-based cART regimen was significantly associated with a 1.35-fold higher AUC for TFV (p=0.0075).

Addition of any one of the remaining unselected candidate predictor variables listed in the Methods section under "Predictor variables" resulted in less than 6% change in the estimated effects shown in Appendix B, **Tables 3** and **4**.

DISCUSSION

Although tenofovir is one of the most commonly used antiretroviral agents in both HIV treatment and pre-exposure prophylaxis settings, limited information is available on the factors that influence its pharmacokinetics under conditions of actual use and in diverse populations. This study examined factors associated with TFV exposure at steady state in a relatively large sample of HIV-infected women who were taking the drug as part of their prescribed cART regimens. The study participants were highly varied in terms of age, race, comorbid conditions, concomitant medications and body habitus, similar to patients in clinical practice. We found significant inter-individual variation in plasma drug levels, and pharmacokinetic parameters (Appendix A, **Figure 2**) in this sample, reflecting medication consumption in real-world conditions of actual use. Four common factors were independently associated with greater TFV exposure: older age, pre-existing mild renal insufficiency, lower BMI and concomitant RTV use.

Ritonavir use increased TFV levels, a finding that is consistent with previous studies that have noted a 32-50% increase in TFV AUC when TDF is co-administered with ritonavir boosted-lopinavir [48, 67]. This association is particularly relevant as the concomitant use of TDF and ritonavir-boosted protease inhibitors occurs frequently in the clinical setting [68]. A recent analysis in the Adults Clinical Trials Group (ACTG) A5208 study demonstrated that young African women randomized to tenofovir/emtricitabine/ lopinavir/ritonavir had a higher incidence of renal insufficiency compared to women randomized to tenofovir/emtricitabine/nevirapine regimens [69], possibly reflecting higher exposure to tenofovir in the latter group. The most likely mechanism underlying this interaction is due to ritonavir-associated inhibition of specific efflux transporters, particularly the p-glycoprotein (P-gp) or multidrug resistant-1 (MDR1) transporter, leading to an increase in the intestinal absorption of TDF and its subsequent exposure [47, 48, 70].

Our study also demonstrates that lower body mass index is associated with modest increases in TFV AUC. Prior studies have demonstrated that low body weight is associated with decreased clearance of TFV [52] and that higher body weight is associated with increased clearance of TFV [49]. Lower eGFR as estimated by cystatin C measured prior to starting TDF (< 70 mL/min/1.73m^2) was associated with a 35% increase in TFV AUC. Although eGFR < 70 mL/min/1.73m^2 as estimated by creatinine measures was associated with higher TFV exposure,

the link between pre-existing kidney dysfunction and TFV AUC was strengthened by using eGFR calculated with cystatin C measures. Because cystatin C levels are independent of muscle mass, cystatin C is a particularly useful measure in chronically ill, aging populations, or in patients with HIV infection, because artifacts in creatinine-based eGFR estimates can occur in individuals with debility or loss of muscle mass [71, 72].

There was an independent and unique association of age (up to the maximum age of 65 in this group) with TFV exposure in our models beyond the effect of renal function. The independent association between age and TFV AUC was maintained whether eGFR was assessed using creatinine measures or cystatin-C measures, and whether eGFR was modeled as a categorical or continuous variable. Although recent data have suggested that age is associated with increased protease inhibitor plasma concentrations [73] and age has a well-established influence on drug pharmacokinetics [74, 75], this analysis is the first to report an effect of age on TFV exposure, independent of renal function.

Adverse effects of TDF on renal function have been described [21, 76, 77] and dose reductions are currently recommended for patients with marked renal insufficiency. However, no modification is recommended for persons with more modest renal dysfunction [4]. There is relatively little available information on TDF pharmacokinetics in patients with mild renal dysfunction and long term use of the drug. A previous study [78] found that the median AUC was 1.41-fold higher in 10 HIV-uninfected subjects with a creatinine clearance from 50 to 79 $mL/min/1.73m^2$ than in three subjects with creatinine clearance >80 $mL/min/1.73m^2$ and another recent study demonstrated that higher TFV troughs are associated with renal impairment [25]. In the previously-cited analysis in ACTG5208, women in the tenofovir/emtricitabine/lopinavir/ritonavir arm with lower pre-randomization creatinine clearance were at the highest risk of developing renal insufficiency events [69].

Our findings are consistent with these reports, but the longitudinal nature of our cohort uniquely allowed us to model the effect of renal function prior to TDF initiation. We found that common factors can combine to significantly increase TFV levels and that mild renal impairment prior to TDF use can significantly affect subsequent TFV exposure. Of note, our models yielded similar findings when eGFR was made into a dichotomous variable as < versus ≥ 80 or < versus ≥ 90 $ml/min/1.73m^2$ as when an eGFR cut-off of <70 ml/min was used. These findings suggest that mild renal insufficiency prior to TDF use could result in a spiral of increased TFV exposure and subsequent renal injury. The recent trials that led to the approval of elvitegravir/cobicistat/TDF/emtricitabine as a single-pill combination excluded participants with eGFR < 70 $ml/min/1.73m^2$ [79, 80], limiting the generalizability of its findings to real-world HIV-infected populations where mild renal insufficiency is not uncommon [81-83], especially among women [84]. Adjustments of TDF dosing based on exposure measures could enhance the safety profile of this important medication.

The WIHS intensive PK studies demonstrate the feasibility and utility of measuring 12 to 24 hour AUCs in large, unselected and diverse populations under actual-use conditions to determine factors associated with exposure in the clinical setting. One limitation is that this study was performed exclusively in a cohort of women, and the results may not be directly applicable to HIV-infected men on TDF-based therapy. We also have not yet examined inter-patient variability as a function of underlying host genetic characteristics, although a future chapter will explore this specific question. In spite of these limitations, there are several

strengths to this study. Notably, there were a large number of individuals included in the analysis with longitudinal data collected over time, including renal function prior to the initiation of TDF-based cART. The study was also conducted under conditions that are representative of how antiretroviral medications are taken in routine practice. The study used a robust measure of tenofovir exposure as assessed by AUCs from intensive PK studies performed over 24 hours. Finally, renal function was assessed both by cystatin-C and creatinine measures in this study.

In conclusion, concomitant ritonavir use, lower BMI, older age and lower eGFR prior to starting TDF were all associated with higher TFV exposure in a cohort of HIV positive women under conditions of routine clinical use. Estimates of GFR using cystatin C may enhance the evaluation of pre-existing renal dysfunction on subsequent TFV exposure and were used in our models. Clinicians providing care to individuals with HIV should be aware of the impact of these common clinical conditions when using TDF. More studies are needed to identify clinically relevant factors contributing to elevated TFV exposure, the pharmacodynamic relationship between exposure and adverse effects, as well as the genetic factors that may contribute to inter-patient variability of antiretroviral concentrations in real-world practice.

Chapter 2: Higher Tenofovir Exposure is Associated with Longitudinal Declines in Kidney Function in Women Living with HIV

Aim 2: Determine how pharmacokinetic variability, as measured by 24-hour tenofovir area under the time-concentration curves (AUC), predicts subsequent change in kidney function over time, in persons living with HIV who are on tenofovir-based antiretroviral therapy.

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INTRODUCTION

Tenofovir disoproxil fumarate (TDF) is one of the most commonly used antiretroviral drugs for the treatment and prevention of HIV infection. Currently, TDF is a component in several one-pill once-a-day combination antiretroviral therapy (ART) formulations and is recommended for first line ART regimens by international guidelines [85]. As TDF formulations become increasingly available in global settings where the burden of HIV is highest, and as the HIV-infected population ages, it is vital to gain a better understanding of the factors that contribute to TDF toxicity.

Systemic exposure to drug is optimally characterized by pharmacokinetic (PK) methods, but prior PK studies of TDF have been limited to populations that differ in race, sex and the presence of comorbid conditions from large populations of HIV patients [26]. Additionally, these prior PK studies have not examined the relationship between variations in TDF exposure and subsequent kidney function. Determination of kidney function following intensive PK analyses is an important means to determine whether TDF exposure is predictive of subsequent kidney impairment.

While TDF is highly effective and safe in most recipients, its use has been associated with reduced kidney function and proteinuria [13, 17, 18, 20, 21, 86], adverse consequences of treatment that can lead to discontinuation of the drug, and which may not be reversible after treatment discontinuation [13, 18, 87]. Although formulated as TDF to promote oral absorption and tissue delivery, PK studies often measure tenofovir (TFV), the active form of the drug in plasma, or its phosphorylated metabolites. For many drugs, the occurrence of adverse effects is directly related to systemic exposure to the drug (defined as AUC). TFV, like the majority of drugs, demonstrates significant inter-individual variability in plasma drug and trough levels that can be affected by a variety of clinical factors [22, 23, 45, 50, 51]. Understanding the PK of TFV is important for providing insight into whether the degree of drug exposure is associated with toxicity. Inter-individual variability in TFV exposure has been hypothesized to be a determinate of kidney injury, but studies to date examining this relationship have been limited by small sample sizes and short durations of observations, by study populations with limited generalizability, and use of non-validated surrogate assessments of TFV PK [24, 25].

The primary objective of this study was to determine if TFV exposure, as measured by TFV areas-under-the-time-concentration-curve (AUCs) is associated with kidney function over a long period of observation. Our hypothesis was that TFV exposure would be independently associated with a decline in kidney function over time.

METHODS

Study design and population. The Women's Interagency HIV Study (WIHS) is a large, multicenter, prospective cohort study of HIV-infected women and at-risk HIV-uninfected women in the United States [53], ongoing since 1993. The WIHS is representative of U.S. women living with HIV in terms of demographic and clinical parameters [53]. The "WIHS Intensive PK Study," has been described previously [58, 59] and enrolled 480 HIV-infected women on varying ART regimens. This intensive PK assessment, conducted from 2004-2008, included 12- to 24-hour sampling of antiretroviral plasma levels after administration of a dose witnessed by study team members. Participant data were included in this analysis for women who reported steady-state use of TDF (that is, TDF use for at least six months prior to PK evaluation), underwent 24-hour intensive PK sampling for plasma TFV levels and had at least one estimated glomerular filtration rate calculated by serum creatinine (eGFRcr) from a WIHS visit after the PK study. Participants were not excluded for ongoing recreational drug use. Laboratory studies, physical examinations, demographic information, self-reported ARV adherence and other characteristics were obtained every six months from WIHS participants as part of participation in the overall prospective cohort study. Institutional review boards at all participating institutions approved the study and informed consent materials. Written informed consent for the PK study was obtained from each study participant. Participants were followed (and included in this study) even if they discontinued TDF, and they were only censored at loss-to-follow-up.

Intensive PK protocol methods. Pharmacokinetic protocols were conducted in clinical research centers or other facilities associated with collaborating WIHS sites. The TFV measurement procedure has been previously described [45], but important details are included here. Participant's typical diet was determined via phone interview 2-3 days prior to the PK study. That diet was then provided during the PK protocol. Patients used their routine medications during the protocol, and these were recorded by study staff. Plasma samples were drawn at timed intervals over 24 hours for drug levels and processed within 30 minutes of sample collection. All participants received their standard dose of TDF with their other medications (which were recorded) and TFV levels were measured in plasma specimens collected at 0, 4, 8, 15, 18 and 24 hours after witnessed dose. Participants were seen for the PK visit within six weeks of a core WIHS cohort visit, thus temporally linking measures between the WIHS data and PK data. Weight and medication use were collected at both the intensive PK and core WIHS visit, whereas data on comorbidities, HIV RNA level, CD4 cell counts and serum creatinine were collected during the core WIHS visits.

Laboratory procedures. Plasma levels of TFV were determined by liquid chromatography/tandem mass spectrometry (LC-MS/MS) with TDF-d6 as the internal standard [60]. The plasma sample was pretreated with trifluoroacetic acid for protein precipitation before injecting into the Micromass Quattro Ultima LC-MS/MS system as previously described (Waters, Milford, MA) [45, 60]. The assay has been validated from 10 to 1000 nanogram (ng)/milliliter (mL) of TFV with a coefficient of variation $< 15\%$ for quality control samples at low, medium, and high concentrations.

Covariates used for adjustment. As in our prior analysis [45], the following baseline covariates were included in all multivariable models: age, race/ethnicity, body mass index, diagnosis of diabetes mellitus or hypertension, self-reported prior duration of TDF usage, concurrent

ritonavir use, current CD4 cell count (log-transformed) and concurrent HIV viral load (detectable vs. non-detectable, above or below assay threshold, 120, 80 or 50 copies/mL (depending on assay used by WIHS at the time of the relevant visit).

Primary predictor. AUCs were used to estimate TFV exposure over the 24-hour dosing interval (once at baseline); these were calculated for each individual using the trapezoidal rule [64]. For calculation of the AUC, any observations with TFV concentrations below the lower limit of quantification (10 ng/ml) were replaced by 0 ng/ml; 10 individuals had undetectable TFV at baseline at the beginning of the intensive PK study and one individual had a level below the lower limit of quantification at the third measurement.

Outcome. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation using serum creatinine was used to estimate glomerular filtration rate (eGFRcr in mL/min/1.73 m^2) [45, 88]. We analyzed changes in eGFRcr as a continuous outcome, expressed as annual differences in ml/min/1.73m^2 per year over approximately seven years of WIHS follow-up.

Statistical analysis. We stratified participants into three categories based on tertile of TFV AUC, and compared demographic and baseline clinical characteristics using the chi-square and Kruskal-Wallis tests for categorical and continuous variables, respectively. Kernel density estimates were used to construct smoothed density curves to examine distributions of baseline eGFRcr levels, and were compared by tertile of TFV AUC using Levene's test for homogeneity of variance. Multivariable linear mixed effect models were used to evaluate the relationship of baseline TFV AUC with subsequent change in kidney function, with random intercepts and slopes using an unstructured variance-covariance matrix for modeling. Graphical examination of the trajectories found curvilinear changes in eGFRcr. Therefore, we modeled eGFRcr using linear splines, with potentially different slopes during the earlier and later follow-up intervals (0-3.5 and 3.5-7 years). We analyzed tertiles of TFV AUC with tertile 1 (lowest AUC) as the reference category for all analyses. We also evaluated levels of TFV AUC (above vs. below median) and baseline eGFRcr (<90 vs. >90 mL/min/1.73 m^2) jointly, to determine whether the association of TFV AUC with subsequent change in kidney function varied by level of baseline eGFRcr. We used relative risk regression with a modified Poisson approach to examine associations of TFV AUC with incident CKD [89]. We analyzed TFV AUC both continuously (per 10% increase) and categorically (using tertiles). We defined incident CKD using eGFR by creatinine, calculated using the CKD-EPI equation as eGFR<50, <60, or <70 at any two consecutive visits over approximately seven years after baseline. All analyses were conducted using SAS (version 9.4, Cary, NC).

RESULTS

The baseline demographic and clinical characteristics of participants in the study, stratified by TFV AUC tertile, are summarized in Appendix B, **Table 5**. A total of 117 women underwent intensive PK sampling for TFV, but 12 women were excluded from analysis due to missing data (including no eGFRcr) or lack of subsequent measures. The recorded dose for TDF was 300 mg once daily in all of the participants' ART regimens. Among the 105 women who contributed data to this analysis, the mean age was 43 years (range 22-65), 68% were African-American, 13% white, and 20% of other race; 25 (24%) self-identified as Hispanic ethnicity. Women with TFV AUC in the highest tertile were older and had lower median BMI relative to those with TFV AUC in the lower tertiles. Concurrent CD4 cell counts were lower and detectable HIV RNA was

more prevalent among women with TFV AUC in the highest tertile than those with lower AUCs, but these differences were not statistically significant.

We examined the distribution of eGFRcr at baseline, stratified by TFV AUC tertile (tertile 1 has the lowest AUC, tertile 2 had intermediate AUC values and tertile 3 had the highest TFV AUC values) (Appendix A, **Figure 7**). The median eGFRcr at baseline was 104 mL/min/1.73 m^2 in tertile 1, 97 in tertile 2 and 78 in tertile 3 of TFV AUC. The spread of eGFRcr levels was considerably wider among participants with exposures in tertile 3 compared with those in tertile 1 and 2 ($p=0.0021$ by test for homogeneity of variance).

We next examined trajectories of change in eGFRcr over time by TFV AUC tertile (Appendix A, **Figure 8**). Mean eGFRcr was lowest among those with highest TFV AUC at baseline (mean mL/min/1.73 m^2 ± SE: 80 ± 4.3 versus 104± 2.5 for highest versus lowest tertile, $p<0.0001$), and these differences widened over time (year 7: 72 ± 4.9 vs. 105 ± 2.9, $p<0.0001$). The trajectories of eGFRcr among women in tertiles 1 and 2 showed similar curvilinear changes, with increases in eGFRcr apparent during years 0-3.5, followed by decreases in years 3.5-7. By contrast, women with TFV AUC in tertile 3 showed decreases in eGFRcr throughout the entire follow-up period. Analyses confirmed that tertile 3 (during years 0-3.5) had significant larger declines in eGFRcr than tertile 1 (-1.87 per year, $p=0.029$; Appendix B, **Table 6**), while there was no statistically significant difference between tertiles 1 and 2. This more pronounced decline in eGFRcr for women whose TFV AUC was in tertile 3 remained statistically significant after multivariable adjustment for all co-factors. During the latter period (years 3.5-7) significant decreases in eGFRcr occurred for women with TFV AUC values in all three tertiles, ranging from 1.4 to 2.4 per year (all $p<0.01$), and no statistically significant differences were detected across tertiles. Appendix B, **Table 7** shows eGFRcr estimates from multivariate linear mixed models using piecewise linear spline, separated yearly.

We then restricted the analysis to include only those participants who had detectable TFV at the start of the study (n=95), which resulted in nine individuals being removed from tertile 1 and one individual being removed from tertile 3. Results were similar after excluding those with undetectable TFV, in that tertile 1 showed a 2.1 ml/min (95% CI 0.92 to 3.4) average annual increase in eGFR over years 0 to 3.5, and a 1.5 ml/min (95% CI -2.6 to -0.53) average annual decline in eGFR over years 3.5 to 7. In multivariable adjusted analysis, over years 0 to 3.5, both tertiles 2 and 3 had smaller gains on average relative to tertile 1. Being in tertile 2 was associated with a 2.3 ml/min/1.73m^2 difference (95% CI -4.2 to -0.45) and being in tertile 3 was associated with a 3.2 ml/min/1.73m^2 difference (95% CI -5.0 to -1.38), relative to tertile 1. Over years 3.5 to 7, differences in annual change narrowed and were no longer statistically different between tertiles. We also evaluated the association of TFV AUC with incident chronic kidney disease using various eGFRcr (50, 60 or 70 mL/min/1.73 m^2) thresholds (Appendix B, **Table 8**). In unadjusted analysis, each 10% increase in the TFV AUC was associated with a 37% increased risk of eGFR<50, a 30% increased risk of eGFR<60 and an 18% increased risk of eGFR<70 (all $p<.05$). Although we had reduced power due to the small numbers of incident cases, we found that greater TFV AUC remained associated with a 22% increased risk of eGFR<70 after multivariable adjustment. When defined as eGFR<70, rates of CKD ranged from 5.7% in tertile 1 to 29% in tertile 3, resulting in a five-fold increased risk of CKD for those in tertile 3 even after multivariable adjustment. Finally, we examined the effect of stratifying by both TFV AUC and baseline eGFRcr, comparing trajectories of eGFRcr change over time (Appendix A, **Figure 9**). We

first considered persons with eGFRcr<90 mL/min/1.73 m^2 at baseline (panel 9A); the eGFRcr trajectory appeared parallel in the lower (n=15) and higher (n=31) TFV AUC groups although the absolute level of eGFRcr was consistently lower in the higher TFV AUC group. In persons with eGFRcr>90 mL/min/1.73 m^2 at baseline (panel 9B), the initial eGFRcr was similar in those with lower (n=37) and higher (n=22) TFV AUC, but those with higher TFV AUC experienced a greater decline in eGFRcr (p=0.025 for years 0-3.5 and p=0.71 for years 3.5-7). Appendix B, **Table 9** shows eGFRcr estimates from multivariate linear mixed models using piecewise linear spline, separated yearly and stratified by both baseline eGFRcr and TFV AUC.

DISCUSSION

This is the first study to demonstrate that greater tenofovir exposure, measured by intensive 24 hour pharmacokinetic assessment, is strongly associated with more rapid declines in kidney function over time. We observed a significant decline in kidney function over the first 3.5 years of follow-up, which did not normalize by year seven. Higher TFV exposure remained independently associated with more impaired kidney function longitudinally, even after controlling for duration of prior tenofovir exposure, baseline kidney function, and other relevant risk factors that may influence kidney function. The association between eGFRcr estimated at the time of intensive PK and TFV exposure as represented by AUCs in this study could be attributable to the effect of reduced kidney function on TFV clearance or the effect of higher TFV concentrations on kidney function. However, the longitudinal association of higher TFV AUC with a more rapid subsequent decline in kidney function provides strong evidence that greater exposure to TFV can injure the kidney over time.

These data indicate that the extent of TFV exposure may influence the occurrence of kidney injury, which may have implications for dosing and prescribing strategies. Our group and others have identified clinical factors that increase TFV AUC in intensive PK studies including concomitant ritonavir use, pre-TFV impairment of eGFRcr, lower body weight and increasing age [45]. Some have advocated for modifications in TDF dosing, particularly with the use of specific protease inhibitors [46]; given the results of our study, such alternative dosing regimens should be further explored to understand whether they may alleviate subsequent kidney toxicity for individuals on this commonly-used antiretroviral agent. We need a better understanding of the factors that determine the pharmacokinetics and pharmacodynamics of TFV and the extent to which these are modified by changes in dosing. Modification of dosing must be evaluated to insure that control of HIV replication is maintained. Our study has shown that there is tremendous between-person variability in exposure to TFV, despite fixed-dose regimens, a fact that is important in understanding TDF's potential toxicity. Determining TFV AUC in the clinical setting may be impractical, but identifying other strategies to predict toxicity should be explored in future studies. One possibility that has yet to be explored is the validation of single trough levels which have been correlated with total daily TFV exposure.

In persons at risk for progression of kidney disease in the setting of TDF use, other agents can be considered as part of the backbone ART regimen in lieu of TDF. For example, abacavir may lead to greater improvements in albuminuria and proteinuria in persons taking ART compared with those on TDF-based regimens [90]. Other therapeutic approaches may lead to lower rates of kidney injury due to lower systemic exposure to the TDF moiety. One such opportunity could be tenofovir alafenamide fumarate (TAF), a novel prodrug of tenofovir that

has been reported as having potential benefits of lower kidney and bone toxicity without a compromise in treatment efficacy [91-95]. Pharmacokinetic studies demonstrated that a TAF dose of 25 mg (compared to 300 mg of TDF) resulted in an approximate 86% reduction in systemic exposure and a 7-fold increase in the mean concentration of intracellular peripheral blood mononuclear cell TFV-diphosphate [96]. In addition, tenofovir from TDF is renally secreted via organic anion transporters (OAT1 and OAT3), whereas TAF has not been shown to significantly interact with OAT or result in OAT-dependent cytotoxicity *in vitro* [94]. However, it is unknown whether TAF treatment will lead to lower kidney tubular cell levels of TFV than TDF since TFV is a substrate for OAT. This idea has been suggested because the lower systemic exposure to TFV seen with TAF and clinical trials demonstrating lower levels of kidney injury markers for subjects using TAF relative to TDF [91-93, 96]. If it is correct that the mechanism of kidney tubular cell injury is attributable to mitochondrial injury, as supported by in vitro studies [97-101], then intracellular concentrations of drug would then seem to be highly relevant. Longitudinal data in real-world settings will be required to determine whether TAF will be a reasonable therapeutic option for persons on TDF who develop kidney or bone toxicity.

There were several strengths to this study. First, this is the largest study to perform intensive PK measurements in patients on tenofovir-based regimens. We were able to follow these participants for an average of more than seven years after completion of exposure determination, allowing a far more comprehensive assessment of the potential kidney complications of higher levels of TFV exposure. These study results were obtained from an ethnically and clinically diverse cohort comprised mostly of racial/ethnic minority women – a group that has been traditionally under-represented in HIV research [102-104].

There were also some limitations to the interpretation of these data. An important assumption underlying our analysis was that TFV AUC does not change over time. This may not be true, and in the setting of declining eGFRcr, it is possible that TFV AUC may be gradually increasing; this could contribute to further reduction of kidney function in a self-perpetuating cycle. In fact, our data support this, given the cross-sectional associations of lower eGFRcr with higher TFV exposure at baseline. It is therefore possible that our quantification of the relationship between eGFRcr and TFV AUC is under-estimated. Also, we measured only kidney glomerular function, and not kidney tubular function. Tenofovir-associated kidney pathology may be a result of tubular injury [105, 106] but the impact on eGFRcr over longer periods of exposure is not clear. This cohort does not yet have serial measurements of biomarkers of tubular injury, such as interleukin-18, kidney injury molecule-1 (KIM-1) or alpha-1 microglobulin (α1m). More specific and early markers of kidney toxicity would enable better determination of the exact mechanisms underlying TFV-induced injury. We were not able to control for genetic factors that may play a role in determining TFV exposure and may be either confounding or modifying the relationship between TFV AUC and subsequent eGFRcr. A number of polymorphisms have been identified that result in increased intracellular TFV concentration or increased proximal tubular injury [35, 44], but whether these factors or others are relevant to the diverse population in this study is not known. The study was limited to women and therefore the results may pertain to women only, and nearly all the women in this study received co-administration of emtricitabine as part of their antiretroviral regimens and an impact of emtricitabine on kidney function cannot be definitely ruled out. Notably, a higher proportion of individuals in tertile 3 had less than seven years of follow-up in the study; if they

dropped out of the cohort because they were sicker or had worse kidney function, then our estimates for the decline in eGFRcr would be underestimated in this group.

In summary, we present compelling evidence suggesting that higher levels of exposure to tenofovir may partially account for subsequent declines in kidney function over time in a cohort of diverse women living with HIV. There is evidence for more rapid progression of kidney function decline in individuals with higher tenofovir exposure. There is also likely an effect of reduced kidney function leading to higher degrees of tenofovir exposure that may operate in a cyclic feed-forward mechanism. These data support further studies exploring the factors that determine tenofovir pharmacokinetics as well as investigations regarding dosing reduction and novel treatment strategies to optimize drug exposure while minimizing drug toxicity in select high-risk populations.

Chapter 3: Evaluating the Association of Single Nucleotide Polymorphisms with Tenofovir Exposure in a Diverse Prospective Cohort of Women Living with HIV

Aim 3: Determine which single nucleotide polymorphisms (SNPs) contribute to pharmacokinetic variability, as measured by 24-hour tenofovir area under the time-concentration curves (AUC), in persons living with HIV who are on tenofovir-based antiretroviral therapy.

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INTRODUCTION

Treatment for HIV infection has dramatically improved in the last 20 years, but lifelong antiretroviral treatment is still required. Ideally, such treatment would represent a sufficient exposure – that is, an exposure to an adequate drug level to suppress HIV replication while minimizing drug toxicity. A barrier to optimal drug exposure is an incomplete understanding of the factors that contribute to inter-individual variability in drug concentration. There are a variety of factors that affect drug exposure, but pharmacogenetics represents an inherent determinant of exposure that may offer an opportunity to understand an individual's potential for achieving viral suppression and/or developing toxicity.

Pharmacokinetics (PK) collectively refers to the absorption, distribution, metabolism and elimination of a given drug, all of which are key factors in determining an individual's exposure to a compound after administration. A variety of PK studies for different antiretroviral medications have demonstrated that increasing drug exposure is commonly associated with toxicity [107-116]. Tenofovir disoproxil fumarate (TDF) is a nucleotide-analog reverse transcriptase inhibitor with broad activity against HIV and is currently co-formulated in a number of pill combinations. TDF is considered to be a World Health Organization essential medication and is a preferred first-line agent in the treatment and prevention of HIV [11, 12]. One primary concern in the use of TDF is the risk of decline in kidney function over time. Recent studies suggest that variability in TFV (tenofovir, the active drug) PK can partially explain the risk of decline in kidney function [108, 117] and although some work has been done to understand the factors that affect TFV exposure [24, 25, 31, 32, 45, 46, 48, 51, 52, 76], the genetic factors that contribute to TFV exposure are not well characterized.

Pharmacogenetics refers to genetic factors that determine PK and specific adverse responses. Such factors could inform precision therapeutics; that is, maximizing benefit and minimizing toxicity while taking into consideration an individual's risk/benefit and dosing profile. With respect to TDF in the treatment of HIV, pharmacogenetic studies have focused on relatively acute toxicity or genotypes associated with intracellular concentrations, but few studies have investigated how single nucleotide polymorphisms (SNP) may affect exposure to the active component of TDF, TFV [31, 34-37, 44, 118, 119]. Thus, the primary aim of this study, in a large prospective cohort of diverse women living with HIV, was to examine how SNPs in TFV pharmacogenes influence TFV exposure, as measured by 24 hour TFV area-under-the-time-concentration-curves (AUCs). The primary hypothesis was that genes with SNPs specifically implicated in the transport of organic anions would result in higher TFV exposure.

METHODS

Study design and population. The study design is cross-sectional, evaluating the association between the TFV AUC and candidate gene SNPs that were selected for inclusion based upon previous published association with TFV toxicity, metabolism or organic anion transport. The Women's Interagency HIV Study (WIHS) is a large, multicenter, prospective cohort study of HIV-infected women and at-risk HIV uninfected women in the United States [53], operational since 1993. The WIHS is representative of U.S. women living with HIV in terms of age, race/ethnicity, socioeconomic status, concomitant medications and comorbid medical conditions [53]. We previously described the "WIHS Intensive PK Study," [58, 59] which enrolled 480 HIV-infected women on different antiretroviral regimens, from 2004-2008, for 12- to 24-hour sampling of various antiretroviral plasma levels after administration of a dose witnessed by study team members. For the current study, eligible WIHS participants were adult women (≥ 18 years of age) living with HIV who had consented to the study (including separate written informed consent for the WIHS study, the genetic study and the PK study), had used TDF for at least six months prior to PK evaluation, had undergone 24 hour intensive PK sampling, and had samples available for SNP testing. Laboratory measurements, physical exams, demographic information, adherence data and several other characteristics were obtained every six months on participants as long as they remained in the cohort. Follow-up of the cohort is ongoing. Institutional review boards at all participating institutions approved the study, consent and protocol materials, and written informed consent was obtained from each study participant.

Intensive PK protocol methods. PK protocols were conducted in clinical research centers or other facilities associated with collaborating WIHS sites. The TFV measurement procedure has been previously described [45], but important details are included.

Plasma samples were drawn over 24 hours for drug levels under conditions of actual use (including diet and concomitant medications). Participants were seen for the PK visit within six weeks of their core WIHS visit, and data were collected at both visits on weight, comorbidities, HIV RNA level, CD4 cell counts, medication use and renal function. All participants received their standard dose of TDF (300 mg orally once daily) and drug levels were measured in specimens collected at 0, 4, 8, 15, 18 and 24 hours after a dose witnessed by study personnel. Calculation of AUC is outlined below.

Laboratory procedures. Plasma levels of TFV were determined by liquid chromatography/tandem mass spectrometry (LC-MS/MS) with TDF-d6 as the internal standard [60]. The plasma sample was pretreated with trifluoroacetic acid for protein precipitation before injecting into the Micromass Quattro Ultima LC-MS/MS system as previously described (Waters, Milford, MA) [45, 60]. The assay was validated from 10 to 1000 nanogram (ng)/milliliter (mL) of TFV with a coefficient of variation < 15% for quality control samples at low, medium, and high concentrations.

Covariates. The following baseline characteristics were included as covariates in all multivariable models as continuous variables: age (for every 10-year increment) and body mass index (for every 10 percent change, measured in kg/m^2). The following baseline characteristics were included as covariates in all multivariable models as categorical variables: race (self-reported African-American or not), estimated glomerular filtration rate (≥ or < 70 *mL/min/1.73* m^2*,* estimated using the serum creatinine) and concurrent ritonavir use (yes or no). Finally, ancestry informative markers (AIM) were used to estimate individual level biogeographic

ancestry and to minimize bias from stratification [120]. Visual inspection of scatter plots of orthogonal principal components (PC) was used to distinguish the major racial/ethnic groups in the sample (i.e., Caucasian, African, and Hispanic). The first three PCs were selected to adjust for potential confounding due to population stratification, by including them in all multivariate regression models. AIMs and their PCs were available for all participants.

Nucleic Acid Extraction. Genomic DNA was extracted previously for all of the participants recruited for the intensive PK studies. DNA samples were quantified by spectrophotometry and normalized to a concentration of 50 ng/μL.

Gene and SNP Selection. A comprehensive systematic literature search identified genes implicated in TDF absorption, distribution, metabolism and excretion (see section below for specific literature review details). A custom array was designed to interrogate nine ADME "pharmacogenes" (i.e., ATP-binding cassette transporter (*ABC*) *B1*, *ABCC2*, cytochrome (*CYP*) *2B6*, *CYP2C19*, *CYP2D6*, *CYP3A4/A5*, solute carrier transporter (*SLC22A6*), UDP glucuronosyltransferase-1 A1 (*UGT1A1*). Genotyping was undertaken using the GoldenGate genotyping platform (Illumina, San Diego, CA). GoldenGate genotyping array data were processed according to standard protocols using GenomeStudio (Illumina, San Diego, CA). Signal intensity profiles and resulting genotype calls for each SNP were visually inspected and confirmed in a blinded fashion. Tagging SNPs (tagSNPs, defined as an efficient subset of SNPs available within a given gene region that are in high linkage disequilibrium (LD) with unmeasured SNPs) were selected from across coding and non-coding regions of each gene in order to capture the majority of the genetic variability surrounding each gene. TagSNP selection was performed using Snagger [121], which selects tagSNPs that are informative across racial and ethnic groups. In addition, SNPs in three additional pharmacogenes specific to TFV ADME or pharmacodynamics (i.e., *ABCC4*, *ABCG2*, adenylate kinase isoenzyme 1 (*AK1*)) were selected based on literature review. Of the additional literature-driven SNPs from among the three additional candidate genes, each was measured by Sanger DNA sequencing. The *CYP2B6* "Metabolizer" haplotype was constructed as described previously [122].

Literature review. A comprehensive systematic literature search was used to identify genes potentially involved in TDF absorption, distribution, metabolism and excretion – the four factors that define pharmacokinetics. The literature search was conducted as follows:

<u>Step 1</u>: Identify all published studies in which tenofovir PK were studied in relation to genetic variability in either humans or animals. Studies were limited to those in the English and Spanish languages. This search occurred in August 2013 and was updated in March 2014. Search terms included: "tenofovir AND SNP," "tenofovir AND gene," "tenofovir AND genetics," "tenofovir AND pharmacogenomics," "tenofovir AND kidney AND genetics," "tenofovir AND OAT," "tenofovir AND organic anion transporter," "tenofovir AND AUC," "tenofovir AND pharmacogenetics," "tenofovir AND absorption," "tenofovir AND distribution," "tenofovir AND metabolism," "tenofovir AND excretion," "tenofovir AND mutation," "ABCC2," "MRP2," "ABCB1," "MDR-1," "ABCC10," "MRP4," "ABCC4," and "hOAT1." No search restrictions were applied other than the language restriction noted above (e.g., no restrictions on timeframe, in vivo or in vitro, species, etc.). The search was conducted using only PubMed, given that we wanted to link the published results to available genetic information for a given SNP; such

genetic information is collated by the National Institutes of Health in the dbSNP database and imports available studies from PubMed.

Step 2: Each of the searches was saved, and duplicate abstracts were identified and removed, yielding 2644 unique abstracts. These were reviewed and only those that described genetic factors associated with tenofovir PK were retained. Any study that described an evaluation for an association between tenofovir PK and genetics was retained.

Step 3: This review yielded 43 publications, of which 19 were retained because they provided specific genetic information regarding tenofovir PK. This yielded a total of five genes, referred to as our "high-yield" gene targets.

Step 4: In the NIH dbSNP website, we identified all possible genes that are functionally related to the five genes identified in step 3. We considered each of these genes in addition to the five high-yield genetic targets, yielding a total of 11 genes of interest: UGT1A1, SLC22A6, CYP3A4A5, CYP2D6, CYP2C19, CYP2B6, AK4, ABCG2, ABCC4, ABCC2 and ABCB1.

Step 5: We then returned to dbSNP and looked at all of the recorded SNPs in each of these 11 genes and documented any SNPs for which there were any published data, in humans, animals or in vitro, yielding a total of 267 SNPs (includes Tagging SNPs). Of these, 27 could not be sequenced. The remaining 240 SNPs constitute those of interest in this aim.

Given that genetic data had been previously collected in this cohort for a genome-wide association study (GWAS), those SNPs that were sequenced as part of the prior GWAS study were available at the beginning of this study. SNPs that were not included in the GWAS study, but that had been identified through the literature review, were sequenced as follows. A custom array was designed to interrogate each of these additional literature-driven SNPs, as well as tagging SNPs (defined as an efficient subset of SNPs available within a given gene region that are in high linkage disequilibrium (LD) with unmeasured SNPs). Tagging SNPs (TagSNPs) were selected from across coding and non-coding regions of each gene in order to capture the majority of genetic variability surrounding each gene.

DNA Sequencing. Three SNPs were typed by Sanger sequencing. Polymerase chain reaction (PCR) primers were designed using Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/) to amplify a region containing the variation. The resulting PCR product was treated with shrimp alkaline phosphatase and exonuclease I enzymes (ExoSAP-IT PCR cleanup kit, Affymetrix, Santa Clara, CA) using the standard product protocol. The treated PCR product served as the template for the sequencing reaction with BigDye Terminator (Applied Biosystems, Foster City, CA). The sequencing reaction was cleaned with X-Terminator (Applied Biosystems, Foster City, CA) and analyzed on the 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA). The resulting sequencing data were viewed using Sequencher (Gene Codes Corporation, Ann Arbor, MI) to perform genotype calling. The final concentrations of the PCR components were 2.5 mM $MgCl_2$, 0.1 mM dNTPs, 0.025 units of platinum Taq polymerase (Invitrogen, Carlsbad, CA), 2% DMSO, 1X PCR Buffer, 200 µM PCR primers and 10 ng DNA template. The 2 µL reaction was run with the following conditions: 95°C for five minutes, [94°C for 20 seconds, 65°C for 20 seconds (0.5°C

decrease per cycle), 72°C for 45 seconds; 14 cycles], [94°C for 20 seconds, 58°C for 20 seconds, 72°C for 45 seconds; 35 cycles], 72°C for ten minutes. The sequencing reaction consists of final concentrations of sequencing buffer, BigDye Terminator mix (Applied Biosystems, Foster City, CA), 500 uM sequencing primer and PCR product template. The running conditions for the sequencing reaction were: 96°C for one minute, [96°C for 10 seconds, 55°C for 5 seconds, 60°C for 4 minutes; 25 cycles]. Custom array genotyping provided 211 SNPs that passed all quality control criteria described below. TagSNPs were required to be common (defined as a minor allele frequency [MAF] $\geq$ 0.05). SNPs with call rates of <95%, or SNPs, which deviated from Hardy-Weinberg expectations ($p<0.001$) were excluded. Finally, SNPs with fewer than three observations in a given genotypic group (e.g., heterozygous, homozygous rare) were excluded.
Outcome. Area-under-the-time-concentration-curves (AUCs) were used to estimate TFV exposure over the 24-hour dosing interval; these were calculated for each individual using the trapezoidal rule [64]. Any observations with TFV concentrations below the lower limit of quantification (10 ng/ml) were replaced by 0 ng/ml (ten individuals at baseline and one individual for a subsequent level).
Statistical analysis. Linear regression modeling using the SAS mixed procedure with robust standard errors was applied to logarithmically transformed AUC, and predictors' coefficients were back-transformed to produce estimated multiplicative effects on AUCs. Genetic association analyses were conducted in the following manner. The first set of models (Appendix B, **Table 10**) show the effect of each individual SNP on log-transformed AUC over dose when combined with non-genetic factors previously shown to influence exposure. Four genetic models were used to assess each SNP: unstructured, additive, dominant, and recessive. The genetic model that best fit the data, minimizing the p-value, was selected for each SNP. A criterion for selection was the presence of at least three observations in each genotypic group. For race/ethnicity, both genetic (ancestry informative markers) and non-genetic parameters were included. In models that examined the effect of each individual SNP on log-transformed AUC/dose (when controlling for non-genetic factors previously shown to influence exposure), only one SNP met the *a priori* p-value threshold ($\alpha = 0.001$). This SNP was then included in a model and all SNPs were then re-screened to identify the next SNP that met this criterion until no additional SNP is retained in the model. No additional SNPs met the *a priori* criterion ($\alpha = 0.001$), and therefore evaluation of models with multiple SNPs was not pursued further. The linearity assumption was evaluated in models with non-genetic factors [45]. An $\alpha = 0.001$ was implemented as a multiple testing penalty for the following reasons. First, the genes that were selected for study have a higher *a priori* probability of being associated with TFV AUC due to evidence of involvement in TFV metabolism. Second, the SNPs spanning each of the genes are not independent (i.e., highly correlated). Therefore, an $\alpha = 0.001$ was reasoned to be an appropriately conservative threshold. All analyses were conducted using Stata (version 11.2, College Station, TX) and SAS (version 9.4, SAS Institute, Cary, NC). The figures were generated using R (version 3.2.3, Vienna, Austria).

RESULTS

The AUC results from a larger study in this cohort (n=101) have been previously summarized [45]. Ten individuals from this cohort did not have samples for genetic analyses, thus data from 91 individuals were included in the present analysis. The median TFV AUC among the 91

participants was 3408 µg x h/mL (range 1026–9356 µg x h/mL). The median age of participants was 44.5 years (range 22.9-64.9 years). The participants were mostly African-American (n=55, 60.4%) with a median body mass index of 27 kg/m^2 (range 15-62 kg/m^2). Of the 240 SNPs that were assessed in the 91 participants, 29 failed quality control measures, leaving 211 for analysis in each participant (Appendix B, **Table 10**). One SNP in *ABCG2* (which encodes for a membrane transporter), rs2231142, was associated with TFV AUC assuming a dominant model, with rare allele carriers (i.e., AA and CA as compared to CC homozygotes) having 1.51 fold increase in TFV AUC (95% confidence interval: 1.26, 1.81; $p=1.7 \times 10^{-5}$). The estimated fold-effect for each SNP is included in Appendix B, **Table 10**. For rs2231142, 14 of 91 individuals carried one of the rare allele, and one individual was homozygous for the rare allele. Appendix B, **Table 11** summarizes the results of the multivariable model controlling for age (per decade), body mass index (BMI, per 10 percent increase), African-American race, ritonavir use, and whether eGFR was less than 70 mL/min/1.73 m^2*.* Appendix A, **Figure 10** displays a boxplot of the distribution of TFV AUC by number of alleles (0 vs 1 or 2) for rs2231142. Given that rs2231142 met the threshold for inclusion in the model, we subsequently re-assessed each SNP in a model with rs2231142. Controlling for rs2231142, no SNP met the *a priori* inclusion threshold to be included in the final model, but the two SNPs that had the smallest p-values are noteworthy for the resultant change in fold-effects for rs2231142 when they were included in the model. The first SNP, rs1128503 from the *ABCB1* gene, which had p=0.0024 in a dominant model, resulted in an increase in the estimated fold-effect for *ABCG2* rs2231142 (fold-effect 1.64, 95% confidence interval: 1.38, 1.96; $p=3.2 \times 10^{-7}$). The second SNP (*ABCB1* rs10236274), which had p=0.0036 in a dominant model, also resulted in an increase in the fold-effect for *ABCG2* rs2231142 (fold-effect 1.62, 95% confidence interval: 1.34, 1.94; $p=1.6 \times 10^{-6}$). The linkage disequilibrium between *ABCB1* rs1128503 and rs10236274 is weak (i.e., $r^2 = 0.013$, D' = 0.49), indicating that each SNP may represent different risk alleles.

DISCUSSION

In this cross-sectional analysis, nested in a cohort study, we present a comprehensive analysis of SNPs previously associated with acute TFV toxicity, metabolism of TFV, or in the transport of organic anions. We were able to identify a single SNP in the *ABCG2* gene that, when present, was associated with a 1.51 fold increase in TFV exposure as measured by AUC. To our knowledge, this SNP has not been previously implicated in TFV pharmacokinetics or toxicity and therefore represents a potentially novel mechanism for how TFV exposure may vary between individuals taking TFV-based antiretroviral therapy.

TFV is commonly prescribed for both the treatment and prevention of HIV infection. Several studies have identified a variety of clinical factors associated with increased exposure to TFV [24, 45, 46, 48, 49, 51, 52, 78, 123]. After including clinically relevant factors in our model of TFV AUC, the genetic factors accounted for the largest effects – a noteworthy finding because genetic polymorphisms often result in more subtle effects on drug exposure than we have found here. In addition, the previously observed influence of higher eGFR on increasing TFV AUC [25, 45, 123] was not found to be significant in the multivariate models in this study. Taken together, these findings indicate that genetic effects could be more pronounced in the presence of other factors that affect drug exposure. Changes in eGFR may be upstream mediators of an underlying genetic effect, but the lack of significance for eGFR in this study was

likely related to the sampling of a sub-group from the larger cohort. Of note, we observed a similar phenomenon (i.e., a biological interaction between the presence of a polymorphism and clinical factors that affect target drug exposure) in an intensive PK pharmacogenetic study of efavirenz in a similarly diverse sample of HIV positive women [59]. Additional study of the interplay between PK, genetics and concurrent morbidities is warranted.

Prior studies have sought to elucidate the pharmacogenetic factors related to TFV exposure and activity, but these have largely focused on acute kidney toxicity, including genetic polymorphisms associated with Fanconi's syndrome and proximal tubulopathy, proteinuria and changes in glomerular filtration [17, 34, 36, 37, 118, 124-127]. We were not able to reach statistical significance for any of these previously identified SNP associations with our more precise, intensive PK assessment of TFV exposure. This is not surprising, in that prior studies looked at acute toxicity events, and this study sought to understand the impact of genetic polymorphisms on TFV AUC. This disparity in findings from studies of acute versus chronic TFV kidney injury possibly indicates that TFV exposure is not as important in acute kidney injury as other factors such as intracellular TFV concentrations. It should be noted that this analysis was based in an observational cohort, in which treatment is determined by the individual participant's provider, and because study assessments occur twice annually, acute toxicity is not likely to be directly observed, although chronic change in renal function after longer treatment exposure is detected. Thus, this study's results indicate that chronic TFV toxicity, which is associated with the extent of drug exposure [108, 117], may be distinct from acute renal toxicity. The polymorphisms identified in prior studies were from genes involved in anion transport at the level of the kidney, similar to *ABCG2*.

The *ABCG2* gene is located on chromosome 4 and encodes for a protein that is found on the apical side of the proximal renal tubular cell and, as a member of the ABC family of transporters, is involved in the transport of anions into the urine. The specific *ABCG2* gene SNP associated with higher TFV exposure in the current study, rs2231142, has previously been associated with a genetic predisposition for increased circulating uric acid and gout [128-132]. *ABCG2* rs2231142 is the result of a missense mutation and is hypothesized to result in loss-of-function that reduces transport of uric acid from inside the renal proximal tubular cell into the urine, producing higher circulating uric acid levels [129]. This mechanism has been demonstrated *in vitro* for both uric acid and several chemotherapeutic agents [129, 133] and provides a plausible mechanism of action for increased TFV concentration in serum. Previous work on placental tissue has shown that TDF, but not TFV, is a substrate for *ABCG2* [134]. Thus, it is possible that alternative formulations of TFV may avoid this genetically influenced increase in exposure.

There are a number of strengths to this study. We were able to obtain a robust measure of TFV exposure in a cohort of diverse women living with HIV. Pharmacokinetic exposure is often estimated using single measures, but capturing a 24-hour pharmacokinetic profile and directly determining AUC may overcome the individual variability that limits interpretation of single measures of antiretroviral concentration [135]. In addition, we had data on most SNPs in several genes that, *a priori*, could reasonably be associated with TFV pharmacokinetics. Evaluating such a large number of SNPs enabled us to perform the most comprehensive assessment of TFV pharmacogenetics conducted to date, while also controlling for established clinical factors that are associated with TFV AUC [45]. We also considered SNPs that have been

implicated in acute renal toxicity with TFV use, allowing us to assess whether these same factors influence more chronic renal injury, indirectly through AUC.

There are limitations to the interpretation of the results of this study. Nineteen polymorphisms assessed in this study did not have sufficiently high allele frequencies to estimate their associations with TFV exposure (see Appendix B, **Table 10)**. Although understanding these associations is important for developing a complete understanding of the pharmacogenetics of TFV, alleles with very low frequency are less likely to have major clinical impact with respect to determining drug exposure. We did account for multiple comparisons in this study by setting an *a priori* threshold of significance at $\alpha = 0.001$. Although some might have opted for a threshold determined by a traditional significance threshold ($\alpha = 0.05$) divided by the number of comparisons (211 SNPs * up to 4 models per SNP = up to 844 comparisons), yielding a threshold of $\alpha = 0.0000592$, doing so would not have affected the identification of the SNP found in this study. Twenty-four hour AUC measurement does not guarantee that an individual was at steady-state concentration, as individuals may have initiated medication in the days leading up to the research study day (and not reported this to study staff). If this occurred, independently of the SNPs evaluated here, the additional random variation would be expected to have attenuated associations with AUC. Furthermore, a single TFV AUC measurement does not reflect day-to-day variation and such variation is influenced by diet, concomitant medications, adherence and substance use. Finally, intensive PK sampling is challenging in most clinical settings, expensive to conduct, and requires tremendous dedication on the part of patients and providers. Such sampling makes our results difficult to generalize to other settings, but may be helpful in establishing the mechanism by which SNPs affect TFV exposure.

In conclusion, we present here evidence of a novel strong association between the *ABCG2* rs2231142 polymorphism and increased TFV exposure as measured by 24-hour AUC in a large prospective cohort of women living with HIV. Understanding how this SNP may lead to elevated AUC is important. More importantly, understanding whether rs2231142 is an upstream determinant that drives renal toxicity from TFV use, either mediated through TFV exposure or through an independent pathway, will be fundamental in elucidating the mechanism by which TFV leads to kidney injury over time.

Chapter 4: Changes in Urinary Biomarkers over Ten Years is Associated with Viral Suppression in a Prospective Cohort of Women Living with HIV

Aim 4: Determine whether urinary biomarkers change in response to changes in antiretroviral therapy and improvements in immune function in persons living with HIV.

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INTRODUCTION

Human immunodeficiency virus (HIV) infection is associated with an increased risk of developing chronic kidney disease (CKD) [14], and among persons with HIV infection, the development of CKD is associated with increased morbidity and mortality [14, 136, 137]. Urine biomarkers have previously been shown to capture subclinical kidney injury that is not detectable by standard clinical measures, such as serum creatinine-based estimated glomerular filtration rate (eGFR) or urine protein concentrations [19, 138-143]. In addition, several urine biomarkers have shown the ability to distinguish the contributions of specific risk factors for subclinical kidney disease. This attribute is particularly important in HIV-infected persons, because CKD can arise as a consequence of multiple different etiologies, including chronic antiretroviral therapy, HIV infection itself, hepatitis C virus (HCV) co-infection and co-morbid chronic illnesses such as diabetes mellitus and hypertension [14, 45, 71, 86, 108, 117, 136, 144, 145]. Furthermore, the presence of two abnormal alleles for the *APOL1* gene predisposes a subset of African-Americans to more extensive glomerular injury and faster CKD progression [146-149].

Several distinct urine biomarkers have been found to be predictive of progressive kidney decline and incident CKD. Our prior work in the Women's Interagency HIV Study (WIHS) identified biomarkers that reflect different pathophysiologic aspects of kidney injury, which could aid in early detection [19, 38-41, 139]. The strongest associations with declines in kidney function have been observed with the urine albumin to creatinine ratio (ACR), alpha-1-microglobulin (α1m) and interleukin-18 (IL-18) concentrations [19, 38-40]. Elevations of the ACR have been associated with declining kidney function in a variety of diseases, including HIV infection, and are indicative of glomerular injury of the kidney [19, 40]. The α1m protein is a low molecular weight protein that reflects proximal tubular dysfunction when elevated in urine samples [19, 38]. Urine levels of IL-18, a pro-inflammatory cytokine involved in cell-mediated immunity, correspond to the extent of proximal tubular injury [19, 40]. In multivariable analyses with comprehensive covariate adjustment, each of these three markers has been independently associated with faster decline in kidney function and higher mortality risk in both HIV infected and uninfected individuals [19, 38, 39, 41].

A major step in understanding the clinical utility of a kidney biomarker involves establishing whether or not serial measures capture longitudinal changes in kidney health. Biomarkers are of lesser value if they cannot capture dynamic changes in risk over time. Therefore, biomarker research in HIV-infected persons needs to evaluate the long-term

changes in novel urine biomarkers and to understand whether they are responsive to changes in important health status measures, including immune function. In this study, to improve on prior studies that have utilized biomarker measures from a single point in time, we present data investigating changes of three urine biomarkers among women living with HIV over a ten year interval. Our primary hypothesis was that improvements in systemic immune status over ten years would lead to favorable changes in the urine biomarker profile of kidney injury.

METHODS

Study design and population. The WIHS is a large, multicenter, prospective cohort study of HIV-infected women and at-risk HIV-uninfected women in the United States [53], ongoing since 1993. The WIHS has been described elsewhere [53, 150]. At semi-annual visits, participants are interviewed and examined, and serum specimens are collected and stored in a −80°C freezer. During the time of this study, women were enrolled in six U.S. sites (Bronx, Brooklyn, Chicago, Los Angeles, San Francisco, and Washington, DC). The enrolled women are representative of U.S. women living with HIV in terms of demographic and clinical parameters [53]. The specific sub-study of this analysis, known as the WIHS HIV Kidney Aging study, is a nested cohort study designed to evaluate incident kidney disease in the setting of HIV infection [19, 38, 151, 152]. Baseline measures of urine biomarkers were conducted on stored urine samples that were collected between October 1999 and March 2000 (year 0) and follow-up measures were made in a subsequent collection in 2010 (year 10). Among 908 women with baseline measures of urine ACR, IL-18 and α1m, we selected a random sample of those women with available stored urine at year ten to include in this study (n=294). WIHS was approved by institutional review boards at all study sites. This study of kidney injury was also approved by the University of California, San Francisco, San Francisco Veterans Affairs Medical Center, Johns Hopkins University and Yale University committees on human research. All WIHS participants provided written and informed consent.

Predictors. Candidate covariates were selected from the baseline and year ten visits, with the exception of CD4 cell count and HIV viral load, which were modeled in several ways, including baseline, peak, nadir and time-averaged values. Candidate variables that were considered as potential predictors of biomarker changes included: time-averaged HIV viral load (in copies/mL), peak HIV viral load (in copies/mL), most recent HIV viral load (copies/mL), time-averaged CD4 cell count (cells/µL), nadir CD4 cell count during study period (cells/µL), nadir CD4 cell count prior to year 0 (cells/µL), most recent CD4 cell count (cells/µL), diastolic blood pressure (in mm Hg), systolic blood pressure (in mm Hg), history of hypertension (yes or no), HCV co-infection (yes or no), current smoking status (yes or no), past smoking history (yes or no), body mass index (in kg/m^2), waist circumference (in centimeters), age (in years), serum LDL cholesterol (in mg/dL), serum triglyceride (in mg/dL), serum HDL cholesterol (in mg/dL), history of AIDS (yes or no) and race (African-American, Caucasian or other). HIV viral suppression was defined as < 80 copies/mL. We also considered changes from baseline for each continuous variable as candidate predictors.

Outcome. The primary outcome was the change in three separate urine biomarkers, ACR, α1m and IL-18, which were measured at years zero and ten of this study. All urinary kidney injury biomarkers were measured at the Cincinnati Children's Hospital Medical Center Biomarker Laboratory. Urine albumin was measured by immunoturbidimetry using a Siemens Dimension

Xpand plus HM clinical analyzer (Siemens, Munich, Germany). Urine α1m was measured by a commercially available assay (Siemens BN II Nephelometer; Siemens, Munich, Germany). Urine IL-18 was measured using a commercially available ELISA kit (Medical & Biological Laboratories Co., Nagoya, Japan). All urine specimens were in continuous storage without prior freeze-thaw. Laboratory personnel were blinded to clinical information about the participants and specimens were evaluated in random order. Assays were performed in 2010 for baseline samples and in 2015 for year ten samples. We repeated 50 baseline measurements on specimens in 2015 and found no evidence for assay drift or bias in any of the assays. For this repeat testing, the Kendall coefficients of concordance W test statistic (and p-value) were as follows: 0.94 (P<.0001) for IL-18, 0.94 (P<.0001) for urine albumin, 1.0 (P<.0001) for urine creatinine and 0.96 (P<.0001) for α1m. The intra-assay coefficients of variation for the urine measures were: albumin, 5.9%; α1m, 5.2% and IL-18, 5.2%.

Statistical analysis. Demographic and clinical characteristics were summarized descriptively at each study visit (year zero and year ten). We used quantile regression to examine the patterns and distributions of change in each urine biomarker over ten years of follow-up. This method enables comparison of biomarker concentrations at the median, quartiles and other percentiles from baseline to year ten and is particularly helpful in exploring differences in the tails of distributions. Multivariable analysis of variance (MANOVA) was used to model predictors and patterns of change in all three biomarkers simultaneously. We standardized each biomarker (to a mean of zero and a variance of one) so that measures with a larger variance would not have a greater influence on model estimates. Models were constructed using the SAS SYSLIN procedure for seemingly unrelated regression (SUR) [153] using the year ten biomarker level as the dependent variable and the year zero level as a covariate along with other factors of interest. The SUR model uses the correlations among the errors in different equations to improve efficiency of the regression estimates, while enabling potentially different predictors for each dependent variable. Because many of the candidate covariates were inter-correlated, we used the least absolute shrinkage and selection operator (LASSO) method for variable selection [154] (R package glmnet). We then applied Bayesian Model Averaging (BMA) to the LASSO-selected predictors, retaining variables with a posterior probability of greater than 35% (R package BMA).

We next identified patterns of changes in biomarker levels using k-means clustering to partition subjects into distinct groups. Our cluster construction was informed solely by the baseline and year ten levels of each biomarker. We used the SAS FASTCLUS procedure to identify outliers and reduce their effect on cluster centers, using the strict option and cubic clustering criterion to determine the final number of clusters. For each biomarker, clusters were compared over the ten-year study period by plotting percentiles in order to compare relative differences within and between clusters. Finally, we used multinomial logistic regression to identify factors associated with cluster membership, using cluster 1 as the reference group. We used the mlogitBMA package for R [155] to perform Bayesian Model Averaging for multinomial logit models, retaining predictors with posterior probabilities >35%. All other analyses were conducted using SAS (version 9.4, Cary, NC).

RESULTS

Demographic and clinical characteristics of the 294 women at baseline (year zero) and follow-up (year ten) are summarized in Appendix B, **Table 12**. Of note, over ten years, the proportion of women diagnosed with hypertension nearly doubled, with an increase in the prevalence of anti-hypertensive use during this time. The prevalence of diabetes mellitus and HCV coinfection remained stable, and all serum cholesterol parameters (LDL, HDL and triglycerides) were on average, improved after ten years. Use of ART increased over ten years, with correspondingly large increases in CD4 cell count and in the proportion of women with viral suppression. There was very little change in eGFR by creatinine over ten years (97 to 95 mL/min/1.73 m^2).

Appendix A, **Figure 11** shows distributions of urine biomarker levels at baseline and year ten. Median IL-18 concentration declined by nearly 50% over ten years; in contrast, median α1m concentration rose by approximately 50%. Although median ACR levels were similar at baseline and year ten, the distribution of values (10th, 90th percentiles) was somewhat wider at year ten (2 to 92 mg/g) compared with baseline (4 to 43 mg/g). Because the three urine biomarkers showed dissimilar trajectories, we performed a cluster analysis that identified four distinct patterns of biomarker change (Appendix A, **Figure 12**). Cluster 1 appeared to be the healthiest subset, with the lowest levels of IL-18 and α1m throughout the follow-up period. In contrast, cluster 2 had the highest levels of both IL-18 and α1m. Cluster 3 was distinguished by having the highest urinary ACR levels at both time-points and α1m levels that rose during follow-up. Cluster 4 was notable for high baseline IL-18 levels that declined sharply during follow-up.

Next, we performed multivariable regression analysis to identify predictors of biomarker changes. Appendix A, **Figure 13** depicts the relative importance of each candidate variable, which represents the posterior probability that each variable has a non-zero association with each biomarker outcome. Dominant factors for IL-18 changes included higher year ten HIV viral load, higher baseline BMI, higher change in waist circumference, lower year ten CD4 cell count and higher year ten HDL cholesterol. The primary determinants of increases in α1m levels were the nadirCD4 cell count both prior to baseline and during the follow-up period. The most important determinant of increasing ACR values was the year ten diastolic blood pressure.

Using variables selected by the LASSO and BMA procedures, we performed a multivariable-adjusted MANOVA regression analysis of changes from baseline to year 10 in all three biomarker levels (parsimonious model shown in Appendix B, **Table 13**, full model available in Appendix B, **Table 14)**. The selected predictors showed distinct associations with changes in each biomarker. Factors associated with increases in IL-18 were higher BMI at baseline, increasing waist circumference, lower HDL cholesterol at year ten, HCV infection, lower CD4 cell count and higher HIV viral load, both at year ten. Factors associated with increases in α1m included decreasing BMI over the study period, smoking tobacco at baseline, higher DBP at baseline, HCV infection, higher nadir CD4 cell count prior to baseline and decreasing nadir CD4 between year zero and year ten. Finally, factors associated with increases in ACR included higher triglycerides at year ten, higher DBP at year ten, HCV infection and higher HIV viral load at year ten.

We then used multinomial logistic regression analyses to identify factors that were independently associated with each of the clusters depicted in Appendix A, **Figure 11** (with model shown in Appendix B, **Table 15**), using the healthiest cluster (cluster one) as the

reference group. Characteristics significantly associated with greater odds of cluster two membership were current smoking (OR=4.55, *P*<.0001) and a history of AIDS (OR=2.71, *P*=0.0061), while higher time-averaged CD4 cell count was associated with a lower odds of cluster 2 membership (OR=0.51 per doubling of CD4, *P*=0.0059). Current smoking was the only independent predictor of cluster 3 membership (OR=2.23, p=0.033), while other factors showed little association. Compared with cluster one, higher time-averaged CD4 cell count was associated with lower likelihood of being in cluster four (OR=0.56 per doubling of CD4, *P*=0.013) compared with cluster one.

DISCUSSION

In this prospective cohort of women living with HIV, we found that the urine IL-18 concentration is a dynamic biomarker that decreased over ten years of follow-up and appeared to be strongly influenced by changes in HIV viral load. In contrast, α1m levels rose over time, while ACR showed little change on average over the course of ten years of follow-up. This is a novel finding in that each of these biomarkers demonstrated a distinct trajectory in these HIV-infected women over the same time period. Although IL-18 levels declined substantially over ten years, particularly in those women with controlled viremia, this beneficial effect was mitigated by higher baseline BMI, increases in waist circumference, lower HDL cholesterol, higher year ten viral load and lower year ten CD4 cell count, and HCV co-infection. Levels of α1m increased on average over ten years, and similarly, were associated with HCV co-infection, but also higher baseline diastolic blood pressure, smoking and lower nadir CD4 cell count at both baseline and during the study period. Lower BMI at follow-up was also associated with increasing α1m, but the change in median BMI from year zero to ten was from 27 to 28 kg/m^2. Finally, although ACR levels were stable on average and remained low in most patients, they were more likely to increase over time in those with higher triglycerides, higher diastolic blood pressure, higher HIV viral load, and in those with HCV infection.

The decline in IL-18 paralleled improvements in HIV control, and the mechanisms by which this occurs are not established. Because urine IL-18 concentrations have been found to be associated with both the incidence of CKD and mortality in the setting of HIV infection, they have been hypothesized to reflect both ongoing subclinical kidney injury and potentially to be a marker of systemic inflammation. It is noteworthy that IL-18 concentrations decreased substantially during the follow-up period, whereas minimal changes were seen with ACR and estimated GFR [40]. Because HIV replication is known to occur in renal tubular cells [141], the findings of this study may support the proposed mechanism whereby HIV treatment leads to reductions in viral replication and lower local inflammation within the kidney.

The increases in α1m at ten years of follow-up were an unexpected finding. Because CD4 cell counts and viral suppression dramatically improved over the ten-year study period, a pattern for α1m similar to IL-18 might have been expected. The divergent findings for IL-18 and α1m warrant additional investigation. In future studies, we plan to measure additional time-points for the biomarkers in order to evaluate changes related to antiretroviral agents, such as tenofovir. ACR is a strong prognostic marker for morbidity and mortality in WIHS [40, 156-161], yet in this study, it remained relatively stable over ten years despite significant improvements in control of HIV infection. However, over the same period, there was a significant increase in diagnosis and treatment of hypertension. This increase in both the prevalence of hypertension

and its' treatment may have offset the benefits gained from better control of HIV infection. Although this is an appealing hypothesis, hypertension was not a significant predictor of the change in ACR, perhaps because hypertension was effectively diagnosed and treated. Alternatively, the lack of change in ACR over the decade of follow-up may reflect the limitations of ACR for capturing changes in HIV health. Our results suggest that, ACR may be a less sensitive marker of kidney injury in the setting of HIV in the setting of relatively stable kidney function, and IL-18 and α1m may reflect pathophysiologic mechanisms that are more specific to HIV disease. Further work clarifying this question will be required.

All of the changes in biomarkers in this study should be considered in the setting of relative improvements in HIV control. As patients were increasingly treated for HIV during this decade of observation, residual inflammation may have persisted due to ART-related toxicities or complications, and improved survival may have led to longer periods of at-risk time in order to develop chronic diseases that are pro-inflammatory. In contrast to prior work, where IL-18 was primarily driven by HIV viral status [151], here we see an effect of metabolic disease on inflammation. These markers have been helpful in identifying evidence for kidney disease [19, 38-41, 139], and this work provides further evidence that the biomarkers could be valuable tools to monitor preclinical kidney disease status. In the evolution of a biomarker for use in clinical practice, it must be shown to be not only prognostic, but also to be responsive to changes in health status.

There are some limitations to the interpretation of these data. We only measured three biomarkers out of many potential measures that could be indicative of kidney disease onset or progression. These biomarkers were chosen because they were associated with CKD and mortality in prior WIHS studies. This study included only women and therefore may not be generalizable to men. The first urine samples were collected over a decade before measurement; any degradation would lead to a non-differential error in quantification of urine biomarkers, but this is unlikely, given the concordance noted on repeat testing from a sample of 50 specimens. Importantly, there is a possibility of residual confounding not accounted for in the modeling, as well as a survival bias in the cohort and therefore the study sample. We did not focus on the relative impact of tenofovir exposure in this study, which is an important consideration given the results and the population under study. To understand the association of tenofovir exposure with these inflammatory markers, we will conduct measures of the biomarkers after additional years of follow-up and longer cumulative durations of tenofovir use. Lastly, these results need to be validated in an external cohort to confirm our findings.

There are several important strengths to this study. To our knowledge, this is the largest study to date of urine biomarker changes in HIV-infected women. Moreover, we had a long duration of follow-up of an ethnically and clinically diverse cohort. This is particularly important in the setting of understanding disease progression among individuals with different genetic susceptibilities to chronic kidney disease. In addition, we were able to consider a large number of possible predictors in examining what factors contributed most to changes in biomarker levels over time, including status of HIV control and comorbid conditions.

In summary, we present data showing that IL-18 is a dynamic urine biomarker that changes in tandem with control of HIV disease and that α1m may be a biomarker that reflects inflammation arising from a number of pathophysiological mechanisms. In contrast, ACR was relatively static over ten years in this longitudinal cohort. Although further study is required to

understand the effects of antiretroviral drug-associated nephrotoxicity on changes in urine biomarkers, this work opens the door to additional research to understand patterns of biomarker change that may predict the development and progression of CKD.

DISCUSSION AND FUTURE STEPS

The studies presented here provide the basis for understanding the factors that contribute to kidney damage in persons living with HIV who are receiving tenofovir-based antiretroviral therapy. More research will be required to elucidate the mechanisms underlying kidney damage, but the results of the current studies provide a reasonable starting point from which to investigate multiple hypotheses efficiently. While these studies have a number of limitations, the work will inform future directions for investigation. We identified important clinical (increasing age, lower BMI, ritonavir use and lower eGFR at baseline) and genetic (a SNP in *ABCG2*) factors that are associated with higher TFV exposure. We also determined that this increased exposure is associated with more rapid decline in kidney function over time, and identified some patterns in urine biomarkers of kidney injury over ten years of follow-up.

Strengths. There are a number of important strengths to these studies and the study design. First, this is the largest study of tenofovir intensive PK that has been done and that is likely to be done. PK studies are time and resource intensive and they often present logistical challenges that cannot be overcome in many research settings. For example, providing a 24-hour monitored and controlled research environment with meals and study staff available can be challenging without the appropriate infrastructure. Furthermore, the ability to obtain clinical specimens at frequent intervals, as was done for these studies, can also be challenging. On the other hand, this measure of PK drug exposure is extremely robust and the optimal assessment of an individuals' 24-hour exposure to a medication. Given that tenofovir is the most widely used drug used in the treatment of HIV infection globally, and its use continues to be scaled up in sub-Saharan Africa, this work can provide insight into the mechanisms that underlie a potentially important adverse consequence of treatment.

Another important strength of this study is that the population consists of women, the vast majority of whom are from minority populations. This group has been traditionally under-represented in research, particularly in HIV studies [102-104, 123]. Including diverse groups of individuals in studies is increasingly important, given that the HIV epidemic in the US now disproportionately affects African-American women [162]. Women in this study were taking the study product under conditions of routine use. That is, these women had been on steady state of their medication, while also taking medications for other comorbid illnesses, consuming their normal diet and maintaining their normal habits (e.g., exercising, illicit drug use, tobacco use and alcohol use) whether healthful or not. In highly controlled studies that are seeking market approval for drugs like tenofovir, participants are carefully selected, often do not have comorbid medical conditions or even HIV infection and are often homogeneous with respect to sex and race [27-29].

The longitudinal nature of this cohort, with baseline data available prior to tenofovir being available in the US market, is another important strength of these studies. We have baseline data on kidney function in the absence of any possible tenofovir exposure. At each visit, participants self-report their use of tenofovir, providing accurate data regarding duration of use – that is, we ask participants to report their use every six months instead of asking them to recall several years of tenofovir use at a single point in time. Similarly, we have collected

clinical samples every six months, and therefore are able to collect laboratory data (e.g., kidney function parameters) prospectively and monitor changes in key laboratory parameters over time, often many years. These studies also make use of historical laboratory data to complement traditional measures of disease (such as serum creatinine or urine albumin for kidney injury) with novel and contemporary diagnostic measures (such as serum cystatin C or urine IL-18 and α1m levels).

With respect to the genetic information available to us from the results of these studies, we are able to improve upon prior research done in this area in several ways. First, we have been able to evaluate more genes and SNPs with respect to tenofovir PK than has ever been done before. The sample size is also quite large, relative to the vast majority of available studies that have investigated the relationship between SNPs and tenofovir drug levels. This is also the only study to assess the impact of SNPs on tenofovir AUC measured over 24 hours – prior studies in this area have used only a single or a few serum measures of tenofovir. Finally, given the large size of the cohort, we are able to control for a number of covariates while assessing the impact of SNPs on AUC, a step that is important in understanding the impact of genetic predisposition on tenofovir PK.

Limitations. In spite of the many advantages that these studies offer, there are limitations to the interpretation of the data. An obvious limitation is that the studies are being performed in a cohort of women, and the results may not be directly applicable to HIV-infected men on tenofovir-based therapy. Although this was a deliberate choice on our part, this is important to consider. A key assumption that underlies our analysis is that tenofovir AUC does not change over time. This may not be true, and in the setting of declining eGFRcr, it is possible that tenofovir AUC may gradually increase. That is, as a function of worsening kidney disease, the AUC of tenofovir could increase over time. This increase in AUC could then trigger more rapid progression of the underlying kidney dysfunction. Biologically, this is a feed forward mechanism that yields a self-perpetuating cycle of kidney injury. The result of using a fixed AUC at a single point in time and assuming that it is static when, it in fact changes over time, is that our estimation of the relationship between eGFRcr and tenofovir AUC would be underestimated. This is so because if the eGFRcr then led to increased tenofovir AUC, which in turn then led to worsening eGFRcr, and higher tenofovir AUC causes more damage than lower AUC, then assuming a static AUC with a rising eGFRcr would underestimate the effect of AUC on eGFRcr.

A unique aspect of tenofovir administration is that, for this study, it was always co-formulated with emtricitabine, a reverse transcriptase inhibitor used in the treatment of HIV infection. Therefore, an impact of emtricitabine on the outcome cannot be definitely ruled out. Regarding discontinuation of tenofovir, it is possible that those individuals who develop kidney disease have their tenofovir treatment stopped by the clinician who is caring for them. Similarly, if such individuals dropped out of the cohort because they were sicker or had worse kidney function, then our estimates for the decline in eGFRcr would be underestimated in this group. The result of having CKD that is too severe to take TDF would be that those individuals with CKD would have a lower cumulative exposure to tenofovir, and therefore would bias the results toward the null hypothesis of no difference in the risk of incident kidney disease in those taking tenofovir, particularly with respect to aim 2, producing a form of survival bias. To address this limitation, I will be controlling for duration of tenofovir exposure and also will stratify

individuals by baseline kidney function, to see if there is a different trajectory of eGFRcr in those with abnormal kidney function at baseline compared to those with normal baseline kidney function. There is also a possibility that the AUC exposure may not be representative of steady-state pharmacokinetics, particularly for those who have an undetectable drug level at baseline (that is, they have not been taking tenofovir prior to the study) leading to an underestimate of the true steady-state exposure among those who are non-adherent at entry to the study. This would result in differential misclassification of the exposure if kidney disease prevented people from taking tenofovir, but as this is an asymptomatic condition, it is far more likely to be non-differential misclassification of exposure leading to a bias towards the null (i.e., such misclassification would decrease the association between kidney disease and TFV AUC). A final limitation worth considering is that intensive PK sampling is very helpful in understanding the mechanisms that might lead to kidney injury, but such sampling is logistically complicated, expensive and impractical in the routine clinical setting. Therefore, the fourth aim of this prospectus seeks to identify urinary biomarkers that can be used to identify early evidence of kidney injury in a variety of clinical settings, including resource limited settings.

There are limitations with respect to our study of urine biomarkers and their association with immune function as well. We measured only three biomarkers out of hundreds of potential markers that could be indicative of disease progression. These were chosen because they have been found to be associated with chronic kidney disease and predictive of subsequent mortality in cross-sectional studies. However, given that the immune system is complicated and not fully understood, urine biomarkers may reflect immunological phenomena throughout the body and not necessarily at the level of the kidney in the setting of HIV infection. The first urine collections were performed over ten years prior to thawing them for analysis, and although we have no reason to believe there has been substantial sample degradation over time, some degradation of the sample while stored might lead to non-differential changes in quantification of urine biomarkers. Lastly, the results of the urine biomarker study will need to be validated in a different population to confirm their validity in order to understand whether changes in urine biomarkers reflect changes in immune function in persons living with HIV infection.

Future steps. At the time of the writing of this dissertation, the studies for chapters 1 and 2 have been published and chapters 3 and 4 are currently under review. No further enrollment of participants, further data collection or conduct of laboratory procedures will be required to complete these studies. Based on these results, two additional studies are being considered. The first of these additional studies will evaluate whether pharmacokinetic variability, as measured by 24-hour tenofovir AUC, is associated with urinary biomarkers of kidney injury in persons living with HIV who are on tenofovir-based antiretroviral therapy. Study two will evaluate whether the SNPs we identified in chapter 3 are associated with the urinary biomarkers we are testing in chapter 4, in persons living with HIV who are on tenofovir-based antiretroviral therapy. These additional studies will allow us to understand how tenofovir exposure, as well as genetic predisposition, may contribute to earlier signs of kidney injury in persons living with HIV who are taking tenofovir. If genetic testing to screen those who may be susceptible to tenofovir-associated kidney injury is not possible, identifying those with early evidence to suggest possible kidney injury while taking tenofovir, prior to development of overt

kidney damage, will be important. The work from this dissertation, when combined with these two additional studies, will provide the basis for applying for additional funding for intervention based trials or as a bridge to further exploratory work in HIV pharmacokinetics.

As roll out of HIV medications continues to expand rapidly in the developing world setting, millions of people will be put on ART, treatment that has the potential to produce significant drug toxicity. Understanding the factors that contribute to tenofovir-associated kidney toxicity will allow clinicians to minimize risk to patients. Identifying patients at risk of kidney injury prior to the development of sustained kidney injury will provide an opportunity to optimize treatment while minimizing toxicity.

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APPENDIX A: Figures

Figure 1. Actual and projected numbers of people receiving antiretroviral therapy (ART) in low- and middle- income countries by WHO region and in high-income countries across World Health Organization regions, 2003-2015. Source: http://www.who.int/hiv/data/art_2003_2015.png?ua=1 (accessed September 2015 and available open-access).

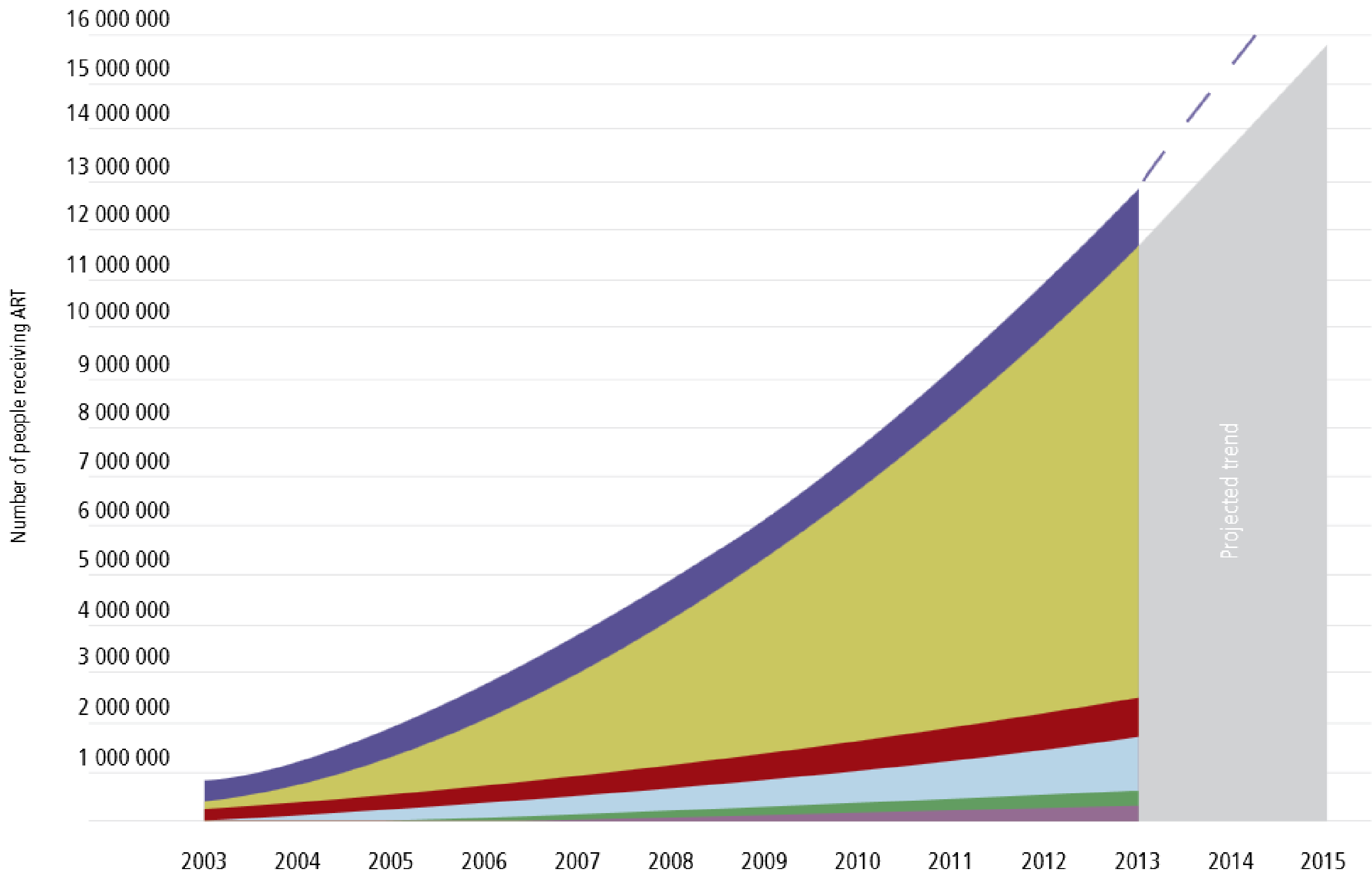


Figure 2. Time-concentration curves for 117 women in 24-hour intensive pharmacokinetic study for tenofovir. Dark line is mean concentration curve.

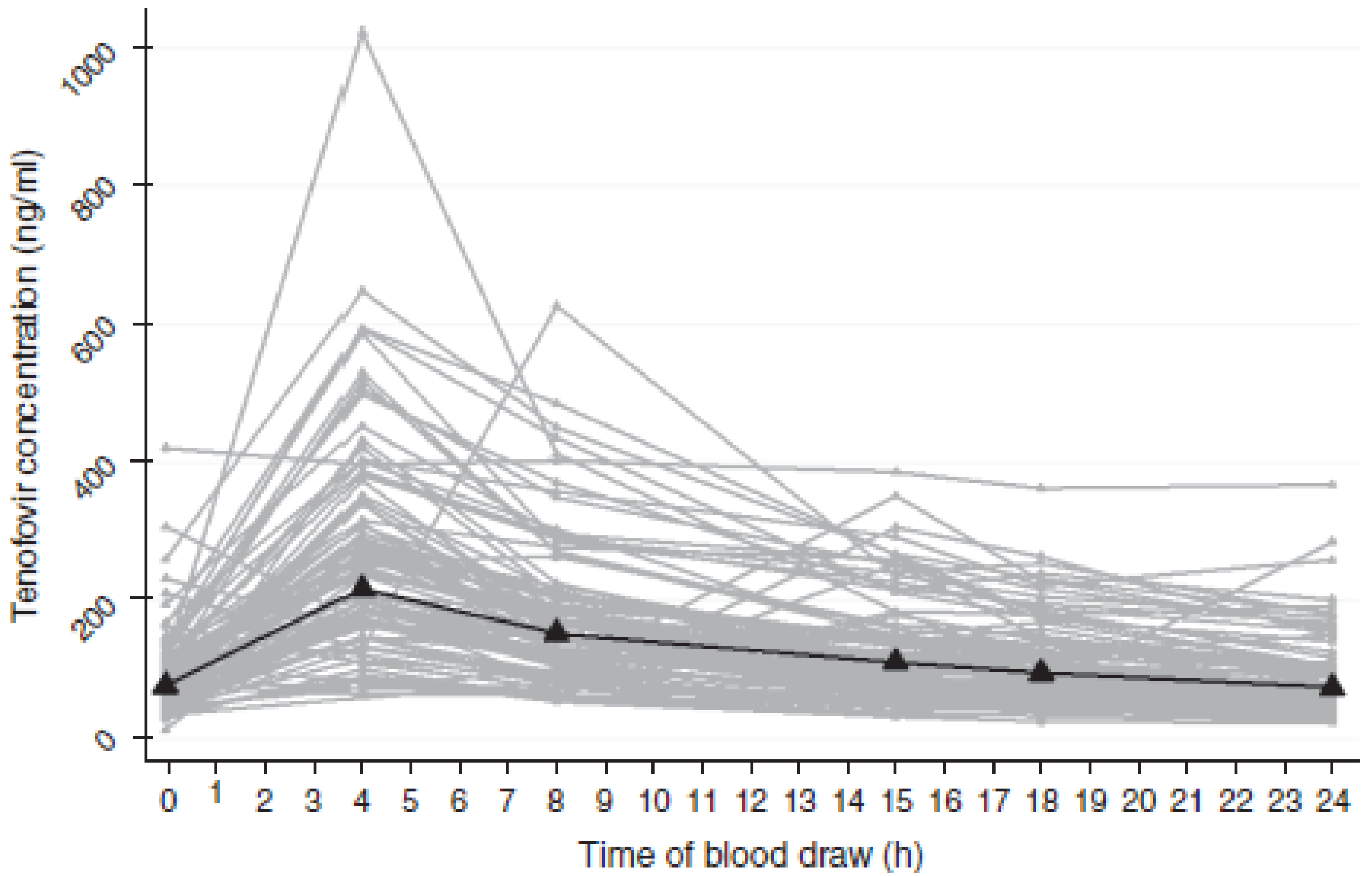


Figure 3. Directed acyclic graph of those factors that contribute to tenofovir area under the time-concentration curves (AUC) in a cohort of women living with HIV on antiretroviral therapy.

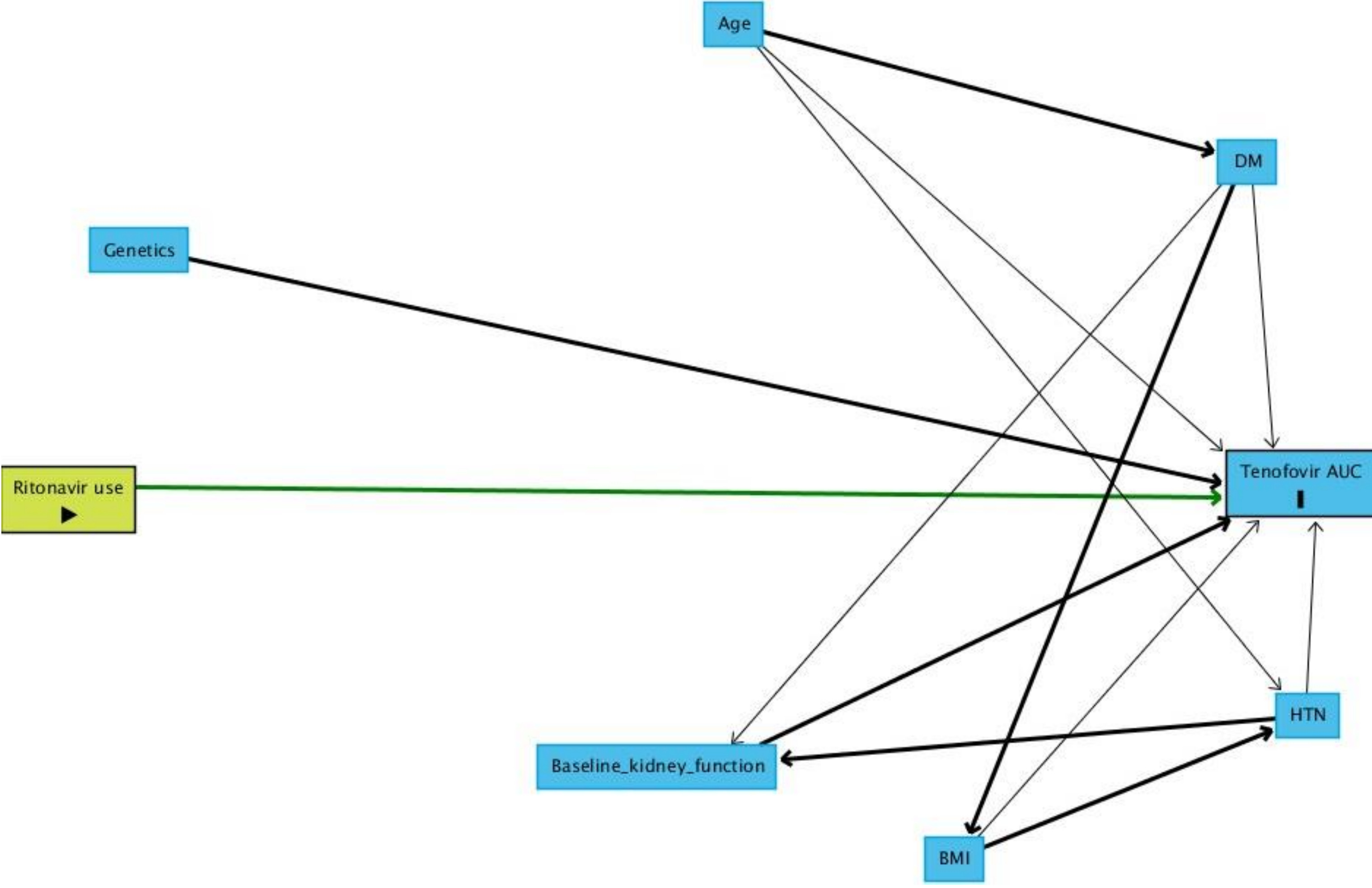


Figure 4. Taken from Hall, *Pediatric Nephrology*, 2013 [43]. Tenofovir toxicity in the proximal tubule cell of the human kidney. Tenofovir is brought into the cell via human organic anion transporter (OAT) and then exits into the kidney tubule via multidrug resistance associated protein (MRP) 2 and MRP4.

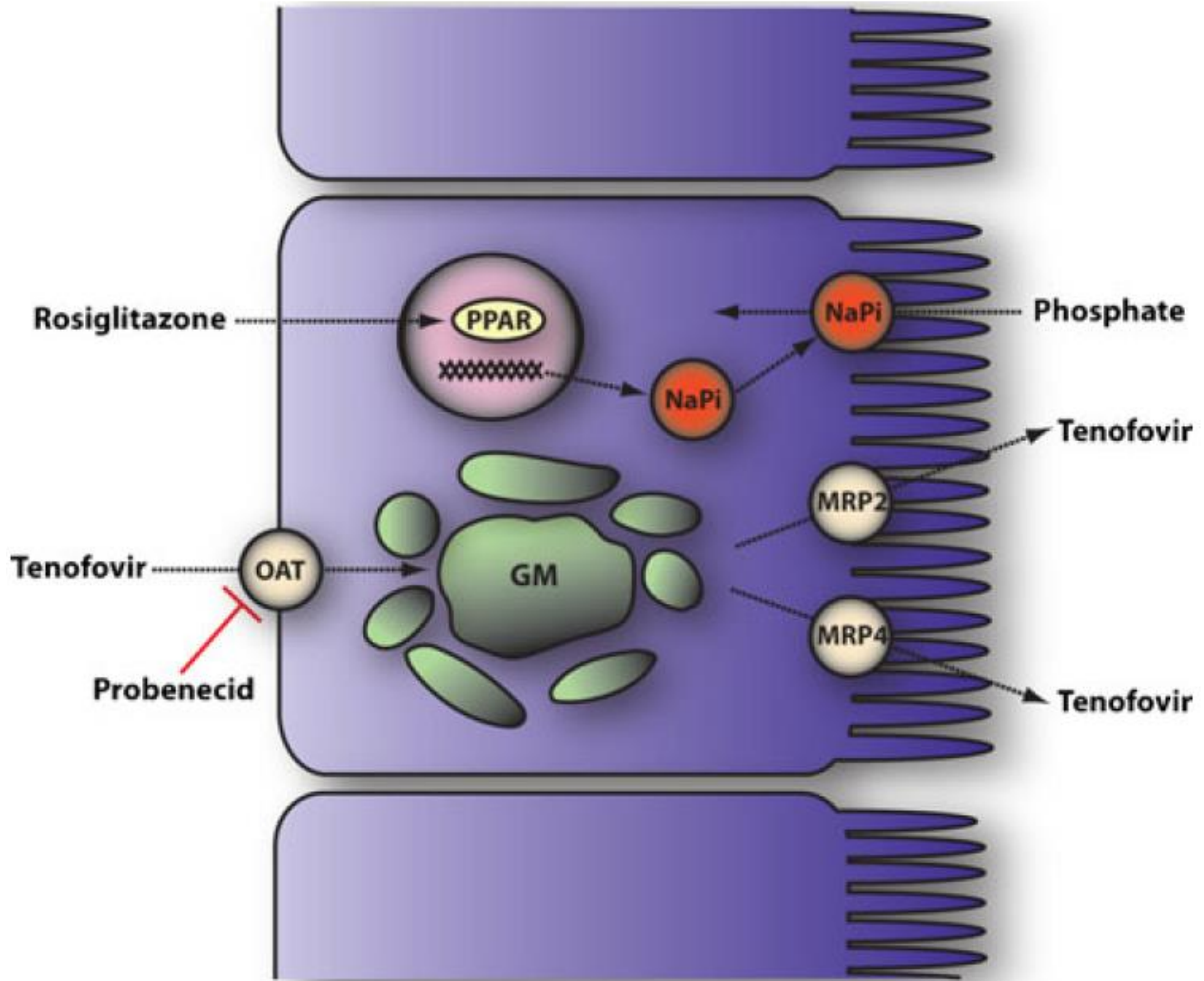
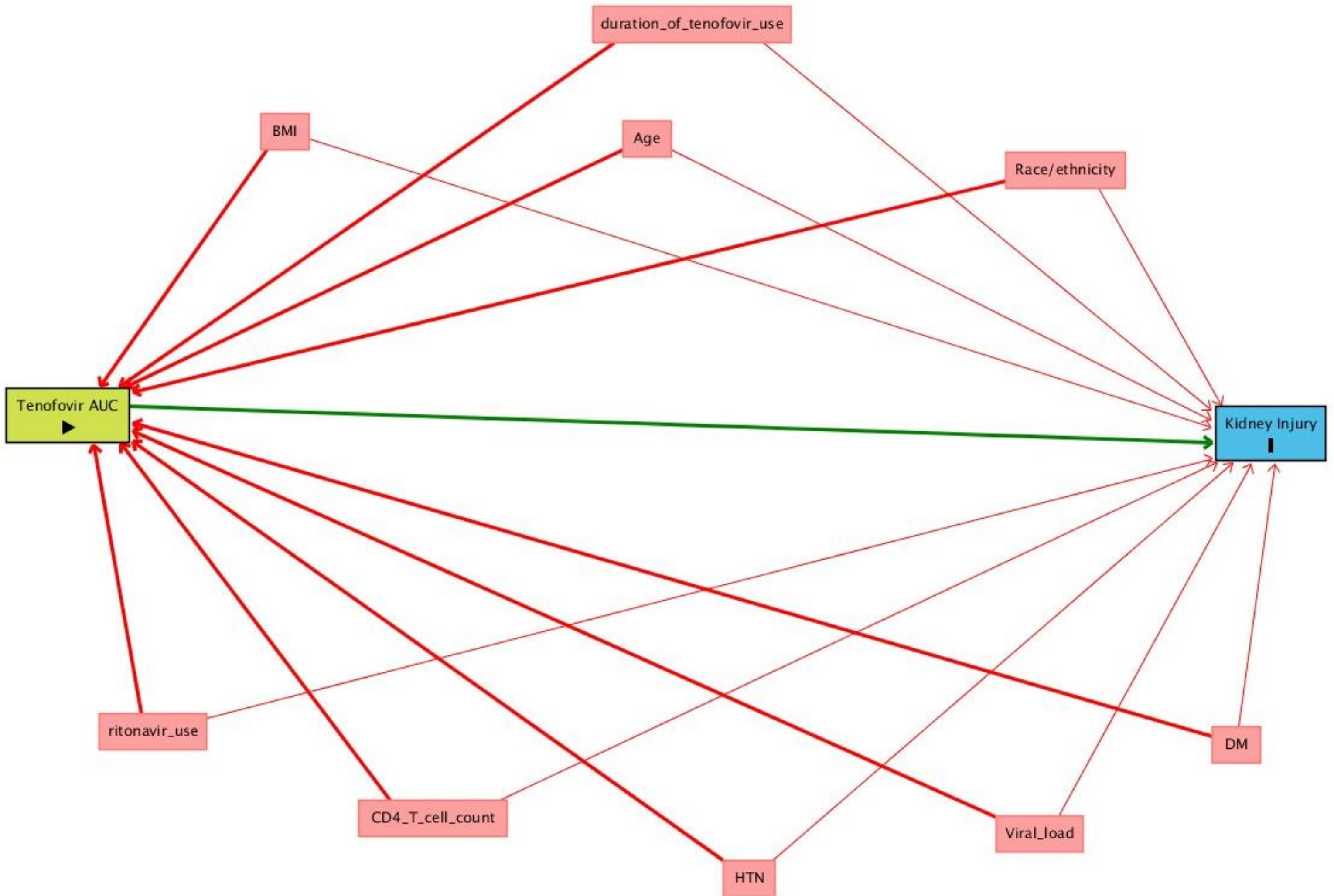


Figure 5. Directed acyclic graph of those factors that affect the relationship between tenofovir area under the time-concentration curves (AUC) and subsequent kidney injury in a cohort of women living with HIV on antiretroviral therapy.



Abbreviations: BMI, body mass index; HTN, hypertension; DM, diabetes mellitus; AUC, area-under-the-time-concentration-curve

Figure 6. Participants in the Women's Interagency Health Study (WIHS) from initiation of the cohort in 1994 through October 2014. Source: WIHS Dossier, October 2014, available at https://statepiaps.jhsph.edu/wihs/. TDF refers to tenofovir disoproxil fumarate use and PK refers to pharmacokinetic assessment.

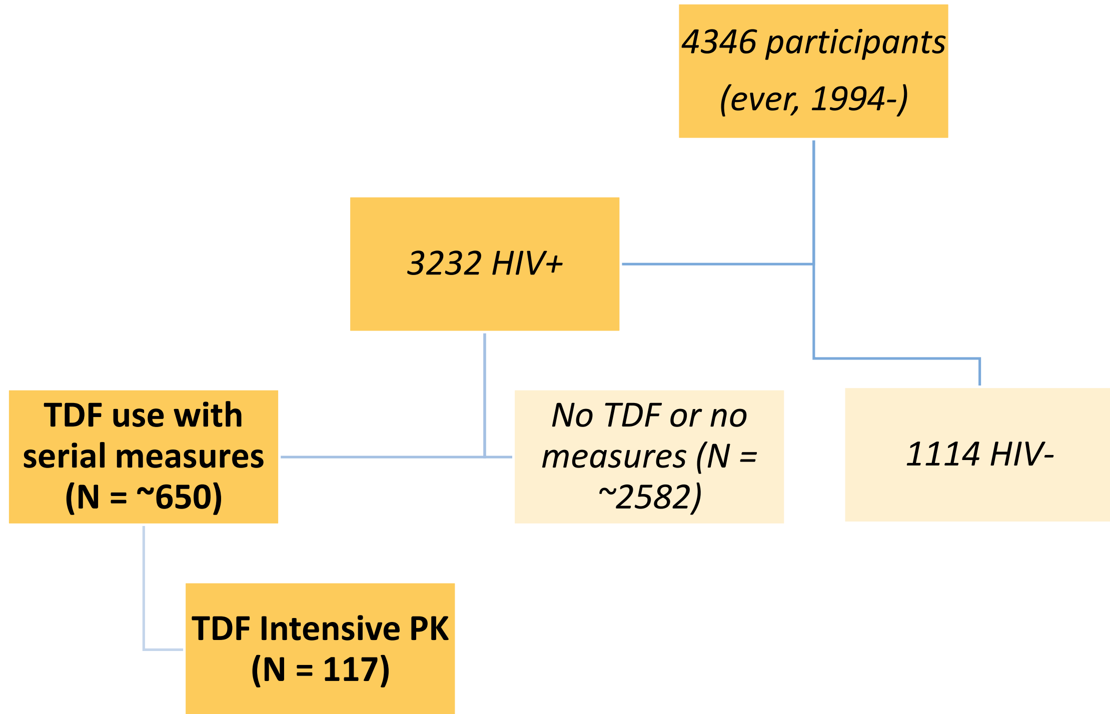


Figure 7. Distribution of baseline eGFRcr (mL/min/1.73 m2) in HIV-infected women, stratified by baseline tenofovir (TFV) area-under-the-time-concentration-curve (AUC) tertile.

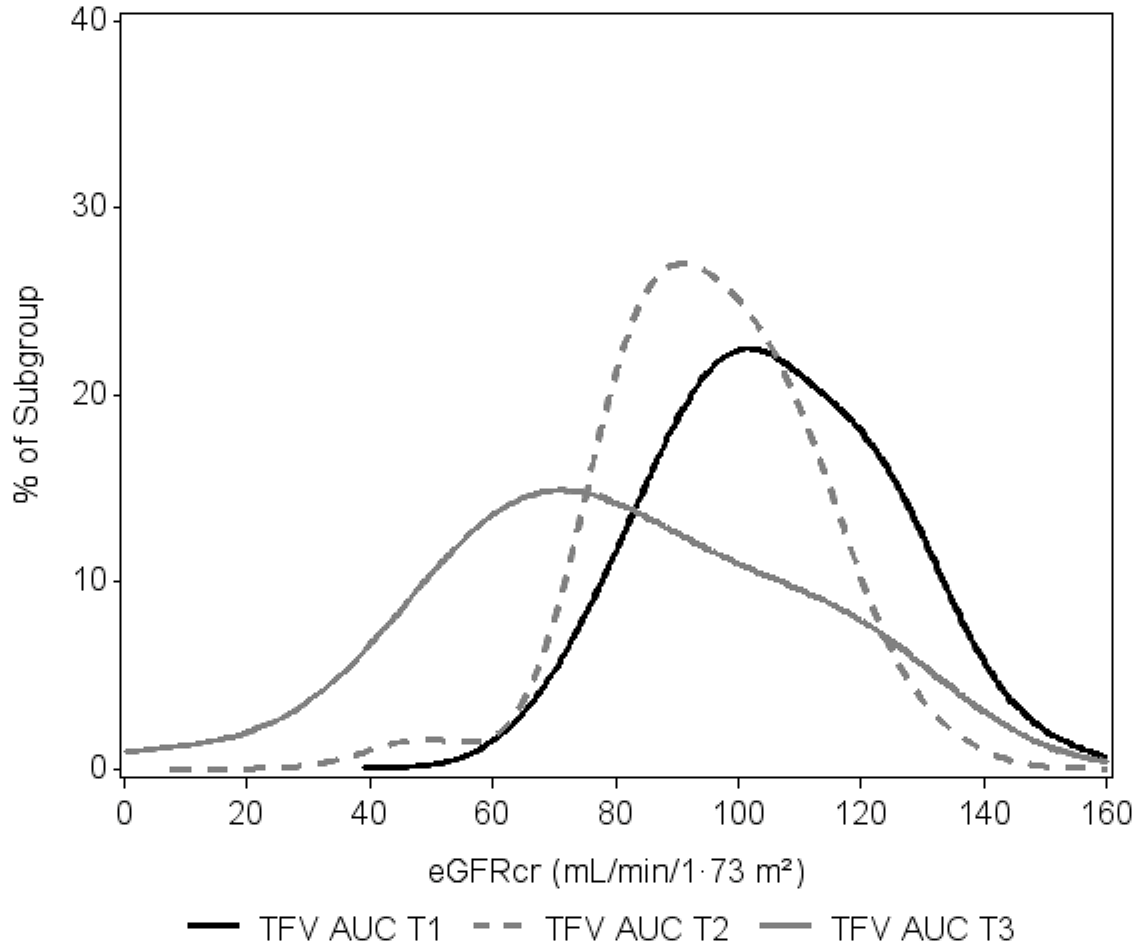
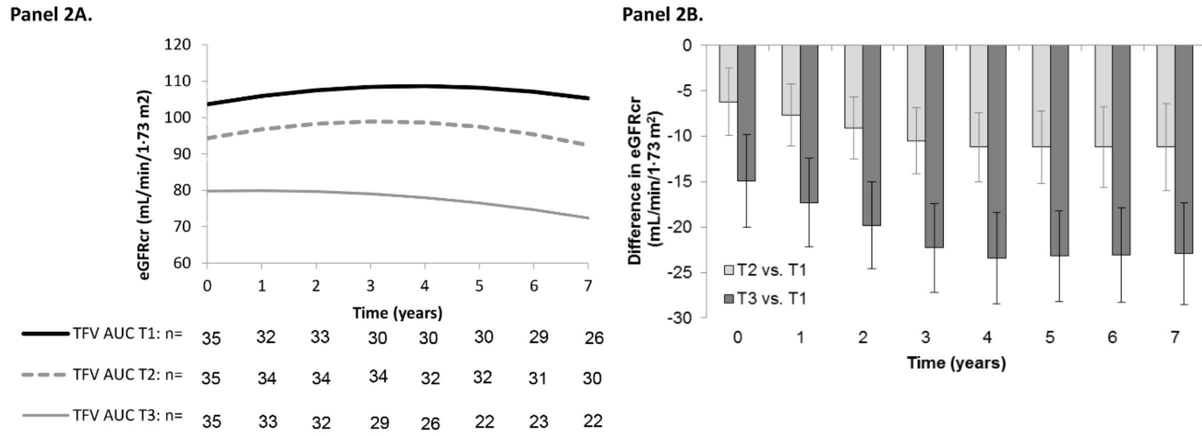


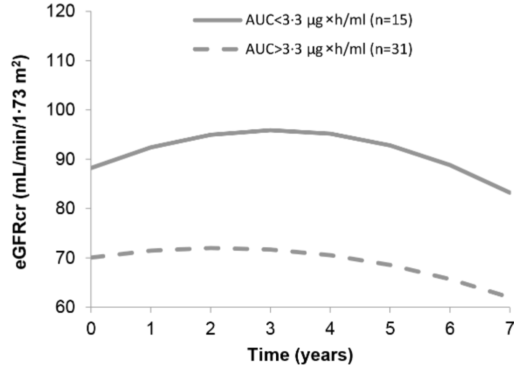
Figure 8. Trajectory of eGFRcr (mL/min/1.73 m2) in HIV-infected women over time (panel A, unadjusted linear mixed model), showing mean. Panel B shows adjusted trajectory of eGFRcr by tertile of TFV AUC with estimates from a linear mixed model with piecewise linear spline, where T1 = TFV AUC tertile 1, T2 = TFV AUC tertile 2 and T3 = TFV AUC tertile 3.



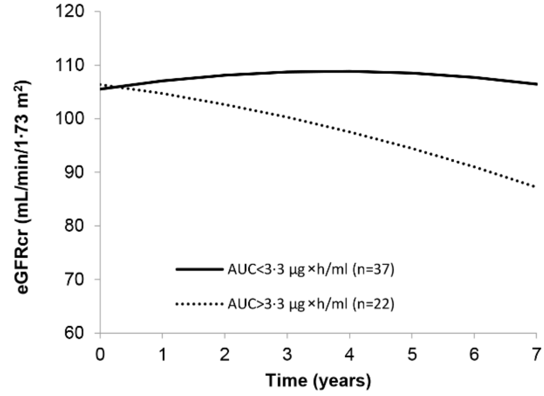
Abbreviations: eGFRcr, estimated glomerular filtration rate by creatinine; TFV, tenofovir; AUC, area-under-the-time-concentration-curve

Figure 9. Trajectory of eGFRcr (mL/min/1.73 m2) over time in patients with baseline eGFRcr < 90 mL/min/1.73 m2 (panel A) or baseline eGFRcr > 90 mL/min/1.73 m2 (panel B) stratified into groups of low TFV AUC (< 3.3 μg x h/mL) versus high TFV AUC (> 3.3 μg x h/mL).

Panel 3A. For baseline eGFRcr < 90 mL/min/1·73 m².



Panel 3B. For baseline eGFRcr > 90 mL/min/1·73 m².



Abbreviations: eGFRcr, estimated glomerular filtration rate by creatinine; TFV, tenofovir; AUC, area-under-the-time-concentration-curve

Figure 10. Boxplot displaying the distribution of 24-hour tenofovir area-under-the-time-concentration-curves (AUC) by number of alleles of *ABCG2* rs2231142 (common homozygotes as compared to heterozygotes and rare homozygote).

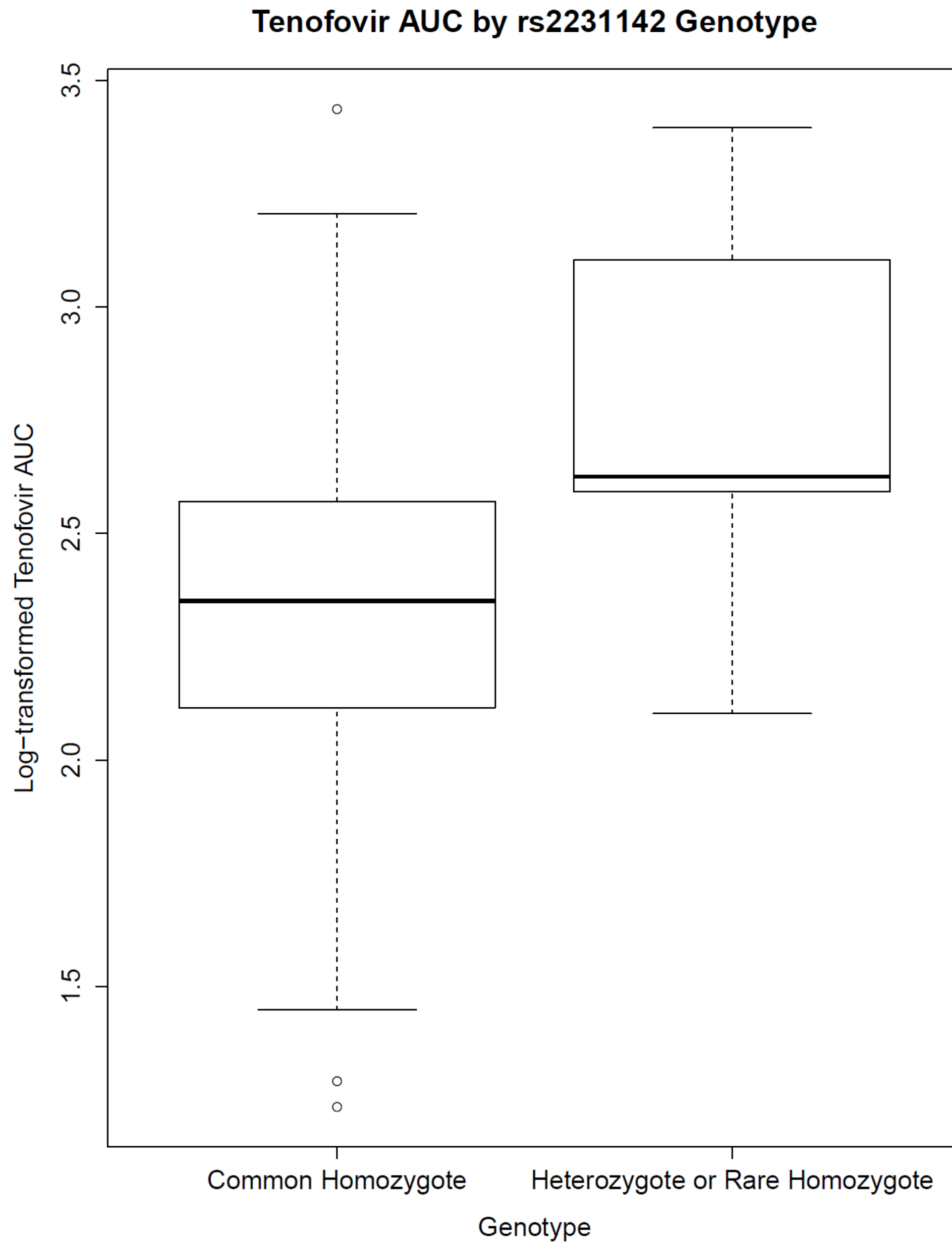
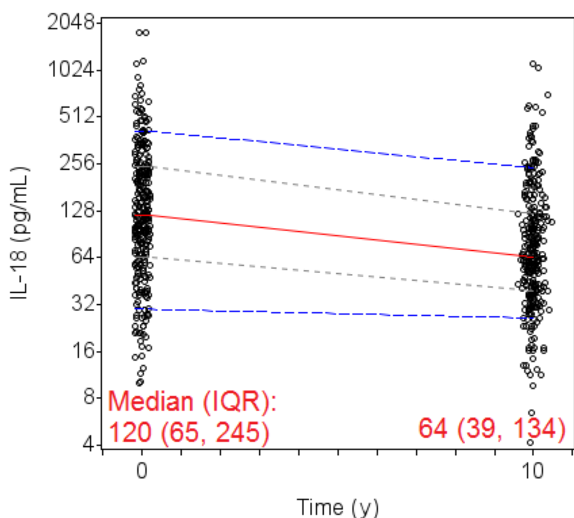
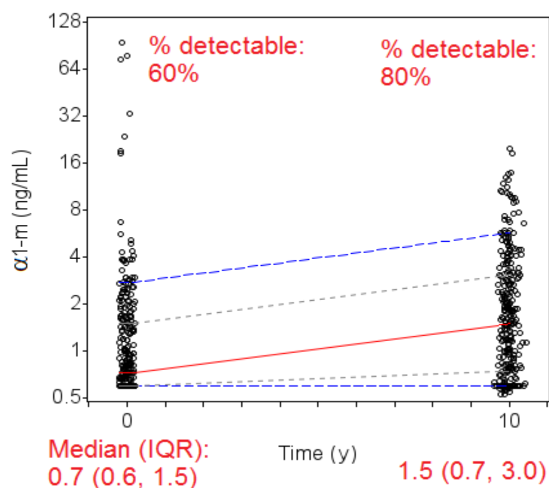


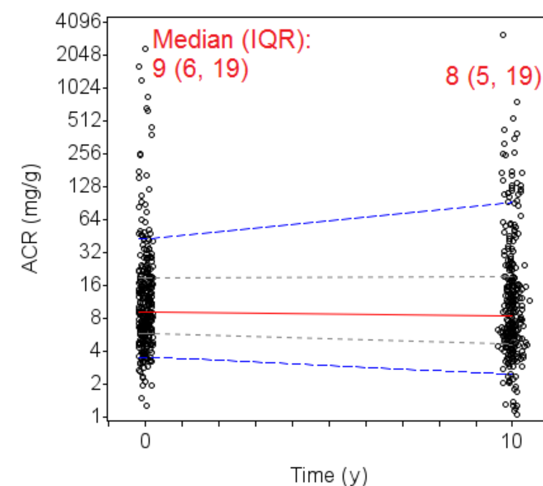
Figure 11. Comparison of urine biomarker level changes in HIV-infected women at baseline and 10 year follow up.

<u>Estimated quantile levels of biomarkers (in pg/mL for IL-18, in ng/mL for α1m and mg/g for ACR)</u>:

---: p90
⋯: p75
–: Median
⋯: p25
---: p10







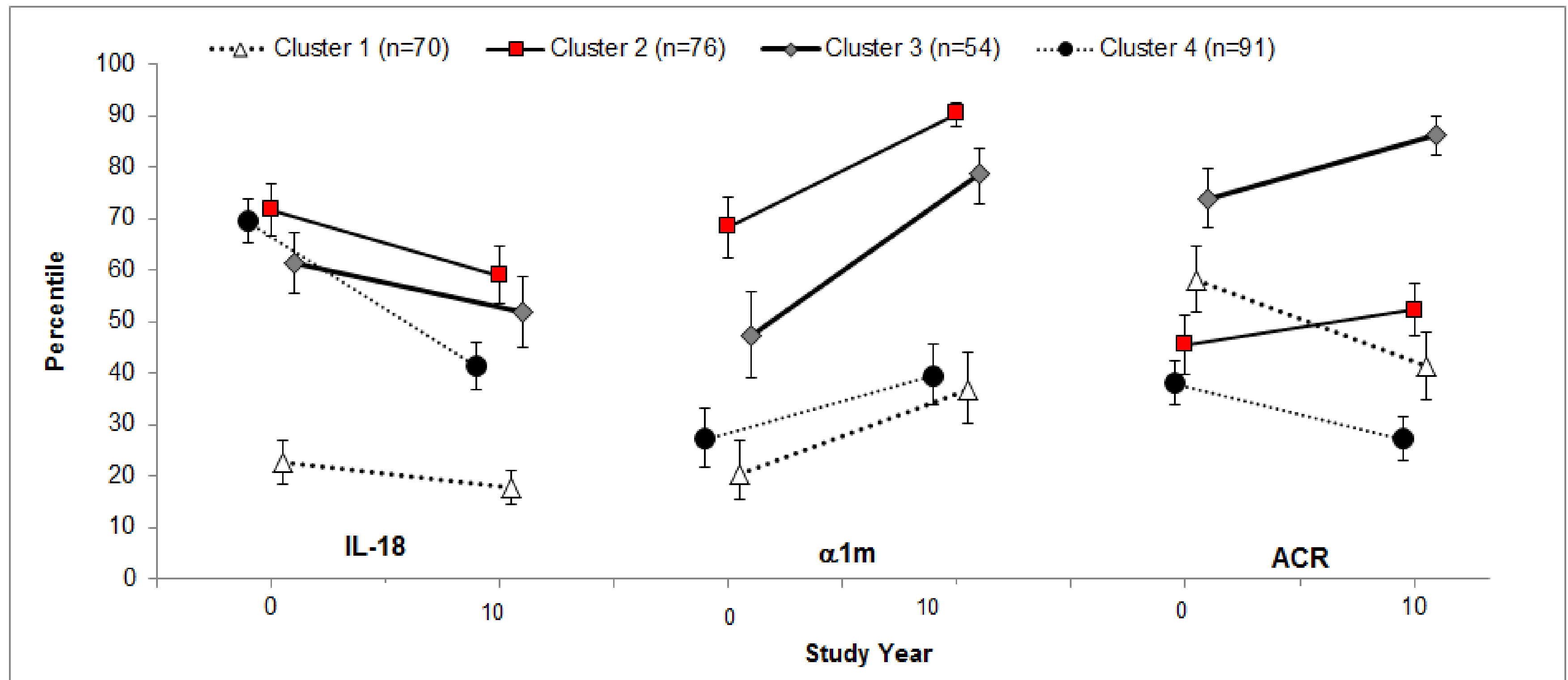
Time	p10	p25	p50	p75	p90
Year 0	30	65	120	245	413
Year 10	26	39	64	123	242

Time	p10	p25	p50	p75	p90
Year 0	0.6	0.6	0.7	1.5	2.8
Year 10	0.6	0.7	1.5	3.0	5.7

Time	p10	p25	p50	p75	p90
Year 0	4	6	9	19	43
Year 10	2	5	8	19	92

Abbreviations: IL-18, urine interleukin 18; α1m, urine alpha-1-microglobulin; ACR, urine albumin:creatinine ratio; y, year; IQR, interquartile range

Figure 12. Estimates (95% CI) plotted represent percentiles of change for each urine biomarker over 10 years of follow-up.

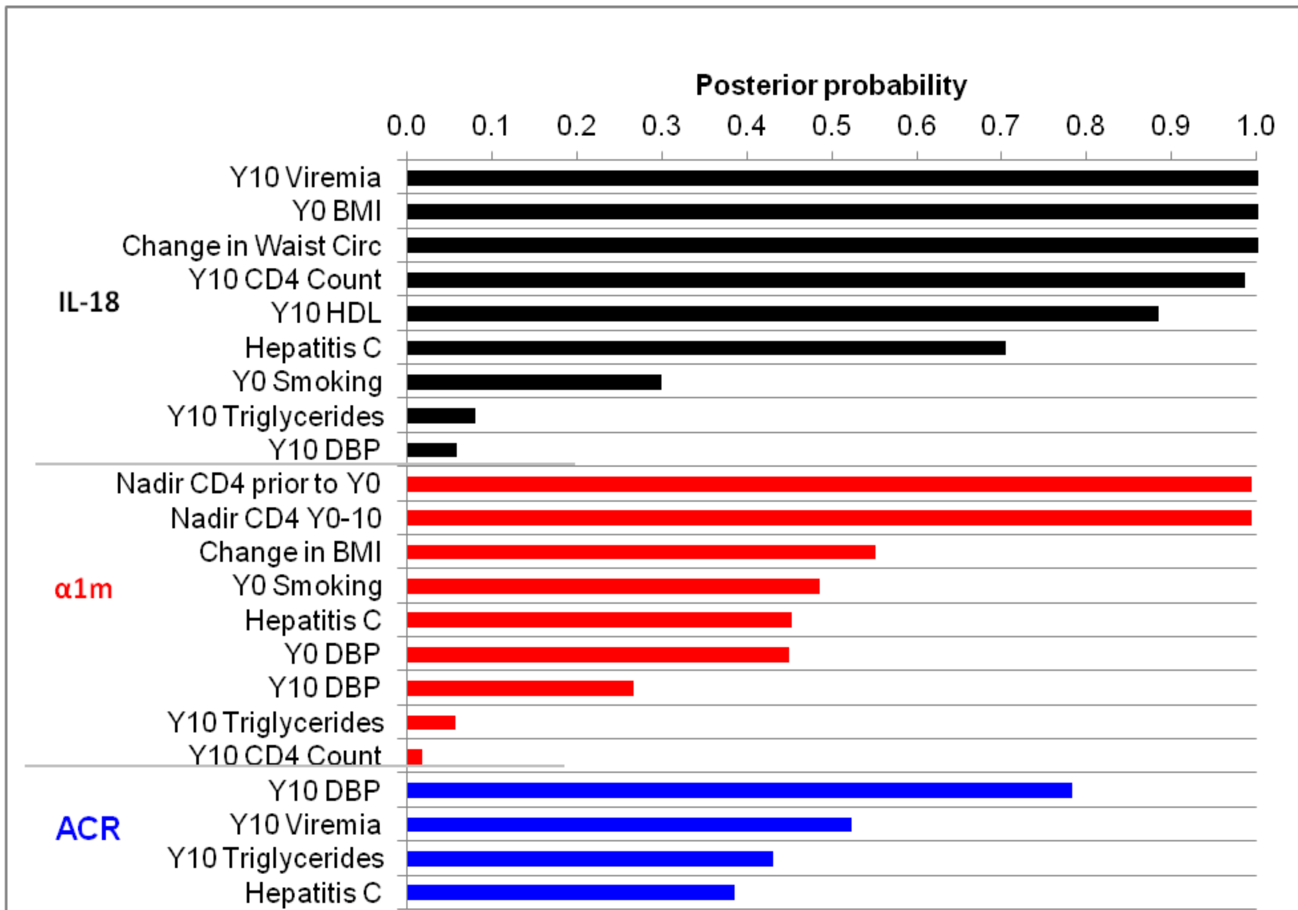


Actual values (median, IQR):

	IL-18 (pg/mL)		α1m (ng/mL)		ACR (mg/g)	
	Year 0	**Year 10**	**Year 0**	**Year 10**	**Year 0**	**Year 10**
Δ Cluster 1 (N = 70)	40 (23, 60)	35 (25, 52)	0.6 (0.6, 0.8)	0.8 (0.5, 1.3)	14 (7, 24)	7 (4, 16)
■ Cluster 2 (N = 76)	207 (111, 349)	111 (66, 229)	1.7 (1.0, 2.7)	4.2 (2.8, 6.2)	8 (5, 14)	10 (6, 16)
♦ Cluster 3 (N = 54)	112 (92, 221)	91 (57, 195)	0.8 (0.6, 1.5)	2.2 (1.4, 3.3)	25 (13, 52)	88 (26, 162)
● Cluster 4 (N = 91)	170 (115, 293)	63 (43, 117)	0.6 (0.6, 0.8)	0.8 (0.5, 1.3)	6 (5, 10)	5 (3, 7)

Abbreviations: IL-18, urine interleukin 18; α1m, urine alpha-1-microglobulin; ACR, urine albumin:creatinine ratio; IQR, interquartile range
Percentiles were computed within the full sample using all available measurements at year 0 and 10, then plotted separately within each cluster. For IL-18 the median change (95% CI in pg/mL) for clusters 1, 2, 3 and 4 were -3 (-29, 17), -60 (-204, 33), -37 (-100, 17) and -94 (-207, -37), respectively. For α1m the median change (95% CI in ng/mL) for clusters 1, 2, 3 and 4 were 0.02 (0.00, 0.53), 2.2 (0.6, 4.3), 0.98 (0.27, 2.5) and 0.04 (0.00, 0.50), respectively. For ACR the median change (95% CI in mg/g) for clusters 1, 2, 3 and 4 were -3.6 (-16, 2.6), 0.8 (-4.2, 8.5), 44 (-20, 115) and -1.5 (-4.1, 0.9), respectively.

Figure 13. Relative importance of variables selected by LASSO and Bayesian Model Averaging.



Abbreviations: IL-18, urine interleukin 18; α1m, urine alpha-1-microglobulin; ACR, urine albumin:creatinine ratio; Y0, baseline year 0; Y10, year 10 follow-up; BMI, body mass index; DBP, diastolic blood pressure

APPENDIX B: Tables

Table 1: Participant characteristics of entire study population (n=101) for chapter 1.

Parameter	**All HIV+ Participants (n = 101)**
Age (years)	*43.1 (21.7-64.9)*
Race (self-report)	
Black	64 (63%)
Hispanic	24 (24%)
White	10 (10%)
Other	3 (3%)
BMI (kg/m^2)*	*28.6 (15.0-62.0)*
Concomitant ritonavir use*	*62 (61%)*
eGFRcr < 70 mL/min/1.73m^2*	*15 (14.9%)*
Hepatitis C antibody positive	37 (37%)
Concomitant diabetes mellitus	26 (26%)
Concomitant hypertension	36 (36%)
Current smoking	54 (53%)
Current alcohol use (vs. abstainer)	36 (36%)
Current crack or powdered cocaine use	16 (16%)
Detectable HIV RNA at time of sampling	36 (36%)
Current CD4 count (cells/mm^3)	409 (4, 1461)

Data above are presented as mean (range) or numbers (percent)
Abbreviations: BMI, body mass index; eGFRcr, estimated glomerular filtration rate by creatinine
*Covariates in italics were retained in final multivariate model due to statistically significant association with TDF exposure

Table 2: Summary exposure metrics for tenofovir in the WIHS intensive PK study.

Tenofovir (n = 101)	AUC (ng·hr·mL-1)	*C*min (ng/mL)	*C*max (ng/mL)	*t*max (hr)	CL/F (mL/hr)
Median	3350	69.7	251	4.1	322
Range	1031-13911	0-363	81.1-1020	0-24	77-1047

AUC, area under the curve; *C*min, trough plasma concentration (all values below the LLOQ of 10 ng/ml were set to 0 ng/ml); *C*max, maximum plasma concentration; *t*max, time of maximum plasma concentration; CL/F, clearance/bioavailability.

Table 3: Multivariate model showing fold-effects on AUC by covariate (renal parameter: CKD EPI, using creatinine prior to visit on TDF), n = 101.

Parameter	Estimate (95%CI) P-value
Concomitant RTV use	↑1.33 (1.11, 1.59), p=0.0020
Per decade of age	↑1.21 (1.08, 1.34), p=0.0007
Black versus non-Black	↑1.04 (0.86, 1.25), p=0.68
Per 10% increase in BMI	↓0.96 (0.93, 0.99), p=0.019
eGFRcr<70 mL/min/1.73m2	↑1.31 (0.95, 1.81), p=0.094

AUC = area-under-the-concentration-curve; CKD epi = the Chronic Kidney Disease Epidemiology Collaboration equation; TDF = tenofovir disoproxil fumarate; RTV = ritonavir; BMI = body mass index; eGFRcr = the CKD-EPI estimate for glomerular filtration rate

Table 4: Multivariate model showing fold-effects on AUC by covariate (renal parameter: CKD EPI, using cystatin C prior to visit on TDF), n = 101, after multiple imputation of cystatin C).

Parameter	Estimate (95%CI) P-value
Concomitant RTV use	↑1.33 (1.12, 1.58), p=0.0014
Per decade of age	↑1.20 (1.09, 1.33), p=0.0003
Black versus non-Black	↓0.97 (0.81, 1.16), p=0.73
Per 10% increase in BMI	↓0.96 (0.93, 1.00), p=0.025
eGFRcys<70 mL/min/1.73m2	↑1.35 (1.08, 1.69), p=0.0075

AUC = area-under-the-concentration-curve; CKD epi = the Chronic Kidney Disease Epidemiology Collaboration equation; TDF = tenofovir disoproxil fumarate; RTV = ritonavir; BMI = body mass index; eGFRcys = the cystatin C estimate for glomerular filtration rate

Table 5. Baseline characteristics of study participants by tertile of tenofovir disoproxil fumarate area-under-the-time-concentration-curve (n=105 total).				
Parameter	**TFV AUC Tertile 1 (n=35)**	**TFV AUC Tertile 2 (n=35)**	**TFV AUC Tertile 3 (n=35)**	**p-value**
TFV AUC range (ng*h/mL)	1031-2640	2646-3922	4009-13911	
Age (years)	41 (31, 47)	42 (34, 49)	47 (44, 51)	0.0004
Race				0.32
African-American	22 (63%)	27 (77%)	22 (63%)	
White	7 (20%)	3 (9%)	3 (9%)	
Other	6 (17%)	5 (14%)	10 (29%)	
Hispanic	11 (31%)	3 (9%)	11 (31%)	0.029
BMI (kg/m2)	28 (25, 36)	29 (26, 33)	25 (22, 30)	0.036
Diabetes mellitus	5 (14%)	11 (31%)	11 (31%)	0.20
Hypertension	12 (34%)	12 (34%)	14 (40%)	0.90
Ritonavir use	13 (37%)	26 (74%)	24 (69%)	0.0040
Duration of prior TDF exposure (years)	1.5 (1.0, 2.0)	1.0 (0.5, 1.5)	1.5 (0.5, 2.0)	0.071
Current CD4 count (cells/μL)	412 (240, 593)	374 (263, 644)	306 (200, 502)	0.097
Nadir CD4 count (cells/μL)	188 (119, 318)	214 (108, 297)	159 (25, 211)	0.099
Detectable HIV viral load	12 (34%)	9 (26%)	16 (46%)	0.24
Baseline eGFRcr (mL/min/1.73 m2)	104 (93, 122)	97 (85, 108)	78 (61, 104)	<0.0001
Years of eGFRcr follow-up	7.3 (6.4, 9.1)	7.7 (7.3, 8.0)	7.0 (4.0, 7.8)	0.014

Continuous parameters are presented as median (IQR) and categorical parameters as N (%).
Abbreviations: TFV = tenofovir; TDF = tenofovir disoproxil fumarate; AUC = area-under-the-time-concentration-curve; IQR=interquartile range; BMI=body mass index; eGFRcr = estimated glomerular filtration rate by creatinine

Table 6. Association of TFV AUC with annual change in eGFRcr (mL/min/1.73 m2) stratified by study follow-up interval (first 3.5 years and second 3.5 years).

Parameter Time Period	TFV AUC Tertile 1	TFV AUC Tertile 2	TFV AUC Tertile 3
Annual Change in eGFR (95% CI):			
Year 0-3.5	1.63 (0.51, 2.7), p=0.0044	1.68 (0.34, 3.0), p=0.014	-0.24 (-1.49, 1.02), p=0.71
Year 3.5-7.0	-1.40 (-2.4, -0.45), p=0.0044	-2.4 (-3.7, -1.12), p=0.0003	-1.92 (-3.2, -0.60), p=0.0051
Unadjusted Difference in annual change vs. Tertile 1 (95% CI):			
Year 0-3.5	Reference	0.048 (-1.69, 1.78), p=0.96	-1.87 (-3.5, -0.20), p=0.029
Year 3.5-7.0	Reference	-0.99 (-2.6, 0.58), p=0.22	-0.52 (-2.1, 1.10), p=0.53
Adjusted* Difference in annual change vs. Tertile 1 (95% CI):			
Year 0-3.5	Reference	-1.41 (-3.2, 0.37), p=0.12	-2.5 (-4.2, -0.76), p=0.0046
Year 3.5-7.0	Reference	0.0079 (-1.61, 1.63), p=0.99	0.17 (-1.56, 1.89), p=0.85

Estimates from linear mixed models. *covariates in adjusted model include: age, race, body mass index, diabetes mellitus, hypertension, ritonavir use, duration of prior tenofovir exposure, CD4 cell count and HIV viral load
Abbreviations: TFV = tenofovir; AUC = area-under-the-time-concentration-curve; T1 = TFV AUC tertile 1; T2 = TFV AUC tertile 2; T3 = TFV AUC tertile 3

Table 7. EGFRcr estimates from multivariate linear mixed models using piecewise linear spline, year over year, assessing the association of TFV AUC with annual change in eGFRcr in mL/min/1.73 m2 (standard error) in WIHS HIV+ participants.

Category	Year 0	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7
TFV AUC T1	104.0 (2.5)	105.7 (2.3)	107.3 (2.3)	108.9 (2.4)	109.1 (2.5)	107.7 (2.6)	106.3 (2.7)	104.9 (2.9)
TFV AUC T2	94.6 (2.9)	96.3 (2.8)	98.0 (2.9)	99.6 (3.2)	99.3 (3.5)	96.9 (3.7)	94.5 (4.1)	92.1 (4.5)
TFV AUC T3	80.0 (4.3)	79.7 (4.2)	79.5 (4.2)	79.3 (4.3)	78.2 (4.4)	76.3 (4.5)	74.4 (4.6)	72.4 (4.9)
UNADJUSTED DIFFERENCES IN eGFRcr								
T2 vs T1	-9.4 (3.8), p=0.015	-9.4 (3.7), p=0.012	-9.3 (3.7), p=0.015	-9.3 (4.0), p=0.023	-9.8 (4.3), p=0.025	-10.8 (4.5), p=0.021	-11.7 (4.9), p=0.020	-12.7 (5.4), p=0.021
T3 vs T1	-24.1 (5.0), p<0.0001	-25.9 (4.8), p<0.0001	-27.8 (4.8), p<0.0001	-29.7 (4.9), p<0.0001	-30.9 (5.1), p<0.0001	-31.4 (5.1), p<0.0001	-31.9 (5.4), p<0.0001	-32.4 (5.7), p<0.0001
ADJUSTED* DIFFERENCES IN eGFRcr								
T2 vs T1	-6.2 (3.7), p=0.092	-7.7 (3.4), p=0.028	-9.1 (3.4), p=0.0096	-10.5 (3.6), p=0.0049	-11.2 (3.8), p=0.0047	-11.2 (4.0), p=0.0073	-11.2 (4.4), p=0.013	-11.2 (4.8), p=0.024
T3 vs T1	-14.9 (5.1), p=0.0047	-17.3 (4.9), p=0.0007	-19.8 (4.8), p=0.0001	-22.3 (4.9), p<0.0001	-23.4 (5.0), p<0.0001	-23.2 (5.0), p<0.0001	-23.1 (5.2), p<0.0001	-22.9 (5.6), p=0.0002

TFV = tenofovir; AUC = area-under-the-time-concentration-curve; T1 = tertile 1; T2 = tertile 2; T3 = tertile 3; *covariates in fully adjusted model include: age, race, body mass index, diabetes mellitus, hypertension, ritonavir use, duration of prior tenofovir exposure, and baseline CD4 cell count and HIV viral load

Table 8: Association of TFV AUC with incident chronic kidney disease by various eGFRcr (mL/min/1.73 m2) thresholds in WIHS participants with HIV.

Outcome	Event Rate	Unadjusted Incident Risk Ratio (95% CI)	Adjusted Incident Risk Ratio (95% CI)
eGFRcr<50			
Continuous AUC:			
Per 10% increase	4/101 (4.0%)	1.37 (1.00, 1.87) p=0.049	n/a
Categorical AUC:			
Tertile 1	0/35 (0%)	Reference	Reference
Tertile 2	1/34 (2.9%)	n/a	n/a
Tertile 3	3/32 (9.4%)	n/a	n/a
eGFRcr<60			
Continuous AUC:			
Per 10% increase	9/97 (9.3%)	1.30 (1.14, 1.48) p=0.0001	1.15 (0.93, 1.42) p=0.19
Categorical AUC:			
Tertile 1	0/35 (0%)	Reference	Reference
Tertile 2	2/34 (5.9%)	n/a	n/a
Tertile 3	7/28 (25%)	n/a	n/a
eGFRcr<70			
Continuous AUC:			
Per 10% increase	12/90 (13.3%)	1.18 (1.05, 1.34) p=0.0068	1.22 (1.02, 1.46) p=0.028
Categorical AUC:			
Tertile 1	2/35 (5.7%)	Reference	Reference
Tertile 2	4/34 (12%)	2.06 (0.40, 10.5) p=0.39	1.50 (0.33, 6.88) p=0.60
Tertile 3	6/21 (29%)	5.00 (1.11, 22.6) p=0.036	5.59 (1.25, 25.0) p=0.025

Estimates from relative risk regression models. *covariates in fully adjusted model: age, race, BMI, diabetes, hypertension, ritonavir, duration of prior TDF exposure, CD4 cell count, viral load.

Table 9. EGFRcr estimates from multivariate linear mixed models using piecewise linear spline, year over year, assessing the association of TFV AUC category and baseline eGFRcr category with annual change and levels of eGFRcr (mL/min/1.73 m2) in WIHS HIV+ participants.

Category	Year 0	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7
C1	105.5 (13.5)	107.1 (12.6)	108.6 (12.4)	110.2 (13.0)	110.6 (13.3)	109.7 (13.4)	108.9 (14.0)	108.1 (14.9)
C2	105.4 (20.2)	104.6 (20.7)	103.9 (22.2)	103.1 (24.5)	101.7 (26.7)	99.5 (28.6)	97.3 (31.0)	95.1 (33.8)
C3	89.1 (20.1)	91.7 (18.9)	94.3 (20.5)	96.9 (24.4)	96.1 (28.7)	91.8 (33.3)	87.5 (38.7)	83.1 (44.6)
C4 (reference)	70.6 (21.2)	71.5 (20.9)	72.4 (21.2)	73.2 (22.3)	72.6 (23.3)	70.5 (24.2)	68.4 (25.6)	66.3 (27.6)
UNADJUSTED DIFFERENCES IN LEVELS								
C1 vs. C4	34.9 (4.2), p<0.0001	35.6 (4.1), p<0.0001	36.3 (4.2), p<0.0001	36.9 (4.4), p<0.0001	37.9 (4.5), p<0.0001	39.2 (4.7), p<0.0001	40.5 (4.9), p<0.0001	41.8 (5.3), p<0.0001
C2 vs. C4	34.8 (4.9), p<0.0001	33.1 (5.0), p<0.0001	31.5 (5.2), p<0.0001	29.9 (5.6), p<0.0001	29.0 (6.0), p<0.0001	29.0 (6.3), p<0.0001	28.9 (6.8), p=0.0002	28.9 (7.4), p=0.0006
C3 vs. C4	18.5 (4.9), p=0.0005	20.2 (4.8), p=0.0001	22.0 (5.0), p<0.0001	23.7 (5.6), p=0.0001	23.4 (6.3), p=0.0007	21.3 (7.0), p=0.0047	19.1 (7.8), p=0.022	16.9 (8.9), p=0.068
ADJUSTED* DIFFERENCES IN LEVELS								
C1 vs. C4	26.8 (4.7), p<0.0001	27.2 (4.5), p<0.0001	27.6 (4.5), p<0.0001	28.1 (4.7), p<0.0001	28.7 (4.8), p<0.0001	29.4 (4.9), p<0.0001	30.2 (5.2), p<0.0001	31.0 (5.5), p<0.0001
C2 vs. C4	28.8 (5.2), p<0.0001	26.6 (5.1), p<0.0001	24.4 (5.2), p<0.0001	22.2 (5.5), p=0.0002	20.9 (5.7), p=0.0006	20.6 (5.9), p=0.0013	20.2 (6.2), p=0.0027	19.9 (6.7), p=0.0056
C3 vs. C4	16.8 (4.2), p=0.0002	17.9 (4.0), p<0.0001	19.0 (4.4), p<0.0001	20.1 (5.0), p=0.0003	19.8 (5.7), p=0.0002	18.0 (6.4), p=0.0092	16.3 (7.3), p=0.035	14.5 (8.3), p=0.092

Tenofovir (TFV) area-under-the-time-concentration-curve (AUC) cutpoint for low vs. high category is based on median of 3.3 μg×h/mL; C1= low TFV AUC & baseline (BL) BL estimated glomerular filtration rate by creatinine (eGFRcr, in mL/min/1.73 m2) >90, C2 = High TFV & BLeGFRcr>90, C3 = Low TFV & BLeGFRcr<90, C4 (reference) = High TFV & BLeGFRcr<90; *covariates in fully adjusted model include: age, race, BMI, diabetes, hypertension, ritonavir use, duration of prior TDF exposure and baseline CD4 cell count and HIV viral load

Table 10. Fold-effect of various SNPs on tenofovir 24-our AUC in 91 women living with HIV.

Gene	SNP	fold-effect (95% CI)	p-value	Model	Common Allele Homozygote	Heterozygote	Rare Allele Homozgote	Freq
ABCB1	rs12673662	0.96 (0.83, 1.11)	0.54	Additive	63	26	2	0.165
ABCB1	rs6946119	1.01 (0.79, 1.29)	0.95	Dominant	72	19	0	0.104
ABCB1	rs1055302	1.06 (0.95, 1.19)	0.28	Additive	45	35	11	0.313
ABCB1	rs1882478	1.10 (0.92, 1.33)	0.30	Dominant	25	49	16	0.450
ABCB1	rs2235047	1.19 (1.01, 1.41)	0.037	Dominant	73	18	0	0.099
ABCB1	rs1045642	0.82 (0.57, 1.19)	0.30	Recessive	50	33	8	0.269
ABCB1	rs4437575	1.16 (0.96, 1.41)	0.12	Dominant	30	38	23	0.462
ABCB1	rs10808071	1.07 (0.91, 1.25)	0.40	Dominant	60	26	5	0.198
ABCB1	rs1002205	1.25 (1.06, 1.47)	0.0097	Dominant	62	24	5	0.187
ABCB1	rs1002204	failed quality control						
ABCB1	rs17149699	1.03 (0.86, 1.23)	0.75	Dominant	60	29	2	0.181
ABCB1	rs6949448	0.92 (0.64, 1.32)	0.65	Recessive	45	42	4	0.275
ABCB1	rs7779562	1.06 (0.92, 1.22)	0.43	Additive	45	39	7	0.291
ABCB1	rs4148745	1.08 (0.90, 1.30)	0.40	Dominant	75	15	1	0.093
ABCB1	rs4148743	1.18 (0.96, 1.45)	0.12	Recessive	35	42	14	0.385
ABCB1	rs2373589	1.09 (0.91, 1.30)	0.33	Dominant	52	34	5	0.242
ABCB1	rs4148740	0.94 (0.77, 1.16)	0.57	Dominant	70	19	2	0.126
ABCB1	rs10225473	0.93 (0.74, 1.16)	0.49	Dominant	73	16	2	0.110
ABCB1	rs7787082	1.10 (0.89, 1.37)	0.36	Dominant	32	42	17	0.418
ABCB1	rs2032583	0.94 (0.77, 1.16)	0.57	Dominant	70	19	2	0.126
ABCB1	rs10236274	1.19 (1.00, 1.40)	0.048	Dominant	69	20	2	0.132
ABCB1	rs4148738	1.06 (0.89, 1.26)	0.52	Dominant	50	37	3	0.239
ABCB1	rs10274587	0.93 (0.74, 1.16)	0.49	Dominant	73	16	2	0.110
ABCB1	rs10248420	1.11 (0.96, 1.27)	0.15	Additive	39	38	14	0.363
ABCB1	rs2235041	1.19 (1.01, 1.39)	0.034	Dominant	76	15	0	0.082
ABCB1	rs1922242	0.80 (0.63, 1.00)	0.052	Recessive	23	54	14	0.451
ABCB1	rs2235046	1.22 (0.89, 1.69)	0.22	Recessive	41	44	6	0.308
ABCB1	rs2235033	0.85 (0.68, 1.07)	0.17	Recessive	27	47	17	0.445
ABCB1	rs2032588	1.12 (0.83, 1.50)	0.46	Recessive	61	26	4	0.187

ABCB1	rs1128503	1.25 (1.06, 1.48)	0.0080	Dominant	51	28	6	0.235
ABCB1	rs2229109	1.02 (0.72, 1.43)	0.93	Dominant	86	5	0	0.027
ABCB1	rs1922241	0.97 (0.66, 1.40)	0.85	Recessive	53	33	5	0.236
ABCB1	rs1882479	0.91 (0.74, 1.10)	0.32	Additive	74	16	1	0.099
ABCB1	rs2235023	1.20 (0.91, 1.56)	0.19	Recessive	51	35	5	0.247
ABCB1	rs4148734	0.91 (0.75, 1.11)	0.36	Additive	70	19	2	0.126
ABCB1	rs1202169	1.22 (0.89, 1.69)	0.22	Recessive	43	42	6	0.297
ABCB1	rs6950978	0.91 (0.76, 1.10)	0.33	Additive	66	18	2	0.128
ABCB1	rs12334183	1.13 (0.89, 1.44)	0.30	Recessive	44	38	9	0.308
ABCB1	rs10264990	0.96 (0.75, 1.23)	0.74	Recessive	53	31	7	0.247
ABCB1	rs1202175	1.20 (0.92, 1.56)	0.18	Recessive	51	34	6	0.253
ABCB1	rs1202172	0.95 (0.85, 1.07)	0.42	Additive	38	40	13	0.363
ABCB1	rs4728705	0.98 (0.78, 1.23)	0.86	Additive	73	17	1	0.104
ABCB1	rs17327442	1.14 (0.97, 1.35)	0.12	Dominant	50	36	5	0.253
ABCB1	rs4148733	1.08 (0.90, 1.30)	0.40	Dominant	59	30	2	0.187
ABCB1	rs1202185	1.20 (0.92, 1.56)	0.18	Recessive	51	34	6	0.253
ABCB1	rs1202184	1.07 (0.88, 1.30)	0.50	Dominant	58	25	8	0.225
ABCB1	rs1211152	0.80 (0.35, 1.82)			89	2	0	0.011
ABCB1	rs1202181	1.20 (0.92, 1.56)	0.18	Recessive	51	34	6	0.253
ABCB1	rs17327624	1.12 (0.93, 1.34)	0.23	Dominant	60	28	3	0.187
ABCB1	rs13229143	1.03 (0.85, 1.26)	0.74	Dominant	51	32	8	0.264
ABCB1	rs3789243	1.05 (0.91, 1.21)	0.49	Additive	19	51	21	0.511
ABCB1	rs1858923	0.90 (0.57, 1.42)	0.64	Recessive	54	31	6	0.236
ABCB1	rs4728707	1.33 (1.11, 1.59)	0.0022	Dominant	76	15	0	0.082
ABCB1	rs17149792	0.86 (0.72, 1.03)	0.11	Dominant	60	29	2	0.181
ABCB1	rs2235074	1.37 (1.12, 1.68)	0.0027	Recessive	19	32	22	0.521
ABCB1	rs9282564	1.05 (0.67, 1.67)	0.82	Dominant	84	5	0	0.028
ABCB1	rs3213619	1.10 (0.88, 1.38)	0.40	Dominant	74	17	0	0.093
ABCB1	rs4728709	1.27 (0.95, 1.70)	0.10	Recessive	37	48	6	0.330
ABCB1	rs4148732	0.84 (0.70, 1.02)	0.071	Additive	76	13	1	0.083
ABCB1	rs13233308	0.80 (0.55, 1.15)	0.23	Recessive	61	27	3	0.181
ABCB1	rs1978095	0.99 (0.69, 1.41)			89	2	0	0.011

ABCB1	rs11975403	0.89 (0.70, 1.14)	0.36	Dominant	86	4	1	0.033
ABCB1	rs2157926	1.51 (1.07, 2.1)	0.020	Recessive	34	54	3	0.330
ABCB1	rs10233247	0.98 (0.75, 1.29)	0.89	Dominant	79	12	0	0.066
ABCB1	rs10275831	0.98 (0.75, 1.29)	0.89	Dominant	79	12	0	0.066
ABCB1	rs10267099	0.94 (0.74, 1.18)	0.57	Dominant	76	13	2	0.093
ABCB1	rs7796247	0.98 (0.75, 1.29)	0.89	Dominant	79	12	0	0.066
ABCB1	rs6465118	1.04 (0.84, 1.29)	0.69	Dominant	64	27	0	0.148
ABCB1	rs17149866	failed quality control						
ABCB1	rs10247258	1.05 (0.87, 1.27)	0.60	Dominant	61	30	0	0.165
ABCB1	HAP_A1	0.92 (0.81, 1.05)	0.22	Additive	40	38	12	0.344
ABCB1	HAP_A2	1.06 (0.89, 1.26)	0.52	Dominant	50	37	3	0.239
ABCB1	HAP_B1	1.09 (0.83, 1.43)	0.54	Recessive	53	32	6	0.242
ABCB1	HAP_B2	1.22 (0.89, 1.69)	0.22	Recessive	41	44	6	0.308
ABCB1	HAP_B3	0.80 (0.63, 1.00)	0.052	Recessive	23	54	14	0.451
ABCB1	HAP_C12	1.15 (1.00, 1.32)	0.055	Additive	54	25	6	0.218
ABCB1	HAP_D4	1.07 (0.88, 1.30)	0.50	Dominant	58	25	8	0.225
ABCB1	HAP_D7	1.20 (0.92, 1.56)	0.18	Recessive	51	34	6	0.253
ABCB1	HAP_E1	0.95 (0.83, 1.10)	0.49	Additive	21	51	19	0.489
ABCB1	HAP_E2	1.42 (1.06, 1.91)	0.020	Recessive	45	42	4	0.275
ABCB1	HAP_E3	0.90 (0.57, 1.42)	0.64	Recessive	54	31	6	0.236
ABCB1	HAP_F1	1.02 (0.78, 1.34)	0.89	Additive	0	12	79	0.934
ABCC2	rs2180989	0.92 (0.80, 1.07)	0.27	Additive	35	42	14	0.385
ABCC2	rs717620	1.15 (0.96, 1.38)	0.12	Dominant	70	18	0	0.102
ABCC2	rs7393105	0.94 (0.73, 1.21)	0.64	Recessive	21	51	19	0.489
ABCC2	rs4148385	0.94 (0.73, 1.21)	0.64	Recessive	21	51	19	0.489
ABCC2	rs2804398	1.05 (0.78, 1.41)	0.76	Recessive	37	43	11	0.357
ABCC2	rs2756109	1.09 (0.89, 1.35)	0.40	Recessive	24	42	25	0.505
ABCC2	rs2273697	1.03 (0.82, 1.28)	0.83	Dominant	68	20	3	0.143
ABCC2	ABCC21346CG	2.00 (1.23, 3.2)			90	1	0	0.005
ABCC2	rs2756112	failed quality control						
ABCC2	rs4148391	failed quality control						
ABCC2	rs2073337	1.18 (0.92, 1.52)	0.19	Dominant	19	52	20	0.505
ABCC2	rs8187674	1.18 (0.91, 1.53)	0.21	Dominant	82	9	0	0.049

ABCC2	rs17112266	0.91 (0.75, 1.09)	0.31	Dominant	75	16	0	0.088
ABCC2	ABCC2IVS1647 GA	1.18 (0.91, 1.53)	0.21	Dominant	82	9	0	0.049
ABCC2	rs11595888	failed quality control						
ABCC2	rs2002042	1.17 (0.86, 1.57)	0.31	Recessive	53	33	5	0.236
ABCC2	rs4148396	1.25 (0.98, 1.59)	0.067	Recessive	35	47	9	0.357
ABCC2	rs4148398	1.10 (0.84, 1.45)	0.47	Recessive	49	35	7	0.269
ABCC2	rs4148399	1.03 (0.76, 1.39)	0.87	Dominant	82	9	0	0.049
ABCC2	rs7898096	1.02 (0.80, 1.29)	0.88	Dominant	75	15	1	0.093
ABCC2	rs7476245	0.93 (0.73, 1.17)	0.53	Additive	67	22	2	0.143
ABCC2	rs17222723	0.79 (0.62, 1.00)	0.053	Dominant	79	11	1	0.071
ABCC2	rs3740066	1.06 (0.94, 1.18)	0.35	Additive	43	38	10	0.319
ABCC2	rs17216282	0.79 (0.62, 1.00)	0.053	Dominant	79	11	1	0.071
ABCC2	rs3740065	0.75 (0.53, 1.08)	0.12	Recessive	60	27	4	0.192
ABCC2	rs8187707	0.77 (0.59, 1.01)	0.056	Dominant	80	11	0	0.060
ABCC2	rs3740063	1.05 (0.88, 1.25)	0.57	Recessive	26	43	22	0.478
ABCC2	rs8187710	failed quality control						
ABCC2	rs7067971	1.06 (0.95, 1.18)	0.31	Additive	45	36	10	0.308
ABCC2	HAP_A2	0.95 (0.80, 1.14)	0.57	Dominant	52	32	5	0.236
ABCC2	HAP_A5	0.92 (0.73, 1.17)	0.52	Recessive	17	52	20	0.517
ABCC2	HAP_B2	1.02 (0.90, 1.16)	0.79	Additive	24	45	22	0.489
ABCC2	HAP_B3	1.06 (0.94, 1.18)	0.35	Additive	43	38	10	0.319
ABCC2	HAP_C1	1.06 (0.95, 1.18)	0.31	Additive	45	36	10	0.308
ABCC2	HAP_C2	0.95 (0.80, 1.13)	0.57	Dominant	22	43	26	0.522
ABCC4	rs1751034	1.16 (0.92, 1.45)	0.20	Recessive	60	24	7	0.209
ABCC4	rs1557070	0.94 (0.79, 1.13)	0.51	Dominant	58	27	6	0.214
ABCC4	rs2274406	1.20 (1.01, 1.42)	0.039	Dominant	35	41	15	0.390
ABCC4	rs2274407	1.16 (0.96, 1.39)	0.12	Additive	63	27	1	0.159
ABCG2	**rs2231142**	**1.45 (1.24, 1.70)**	**0.000010**	**Dominant**	**77**	**13**	**1**	**0.082**
AK1/4	rs1109374	0.92 (0.75, 1.13)	0.42	Recessive	46	41	4	0.269
CYP2B6	rs2054675	0.96 (0.80, 1.15)	0.68	Dominant	44	39	8	0.302
CYP2B6	rs34223104	0.57 (0.39, 0.84)	0.0053	Dominant	87	4	0	0.022
CYP2B6	rs35303484	failed quality control						

CYP2B6	rs6508963	failed quality control						
CYP2B6	rs8100458	failed quality control						
CYP2B6	rs8192712	0.97 (0.77, 1.24)	0.83	Additive	80	10	1	0.066
CYP2B6	rs16974799	1.10 (0.81, 1.50)	0.55	Recessive	44	37	10	0.313
CYP2B6	rs4803417	0.88 (0.66, 1.17)	0.36	Recessive	48	38	5	0.264
CYP2B6	rs36060847	failed quality control						
CYP2B6	rs2279342	0.75 (0.58, 0.96)	0.021	Dominant	54	20	0	0.135
CYP2B6	rs35773040	failed quality control			91	0	0	0.000
CYP2B6	rs3745274	0.96 (0.80, 1.16)	0.69	Dominant	47	36	8	0.286
CYP2B6	rs36079186	failed quality control						
CYP2B6	rs8113200	0.84 (0.66, 1.07)	0.15	Recessive	46	35	10	0.302
CYP2B6	rs2279343	failed quality control						
CYP2B6	rs2279345	0.78 (0.63, 0.97)	0.027	Recessive	44	37	10	0.313
CYP2B6	rs12721649	0.96 (0.81, 1.15)	0.68	Additive	64	26	1	0.154
CYP2B6	rs2306606	0.94 (0.79, 1.13)	0.52	Dominant	41	38	12	0.341
CYP2B6	rs28399499	0.94 (0.79, 1.10)	0.43	Additive	79	11	1	0.071
CYP2B6	rs34097093	failed quality control						
CYP2B6	rs35979566	failed quality control						
CYP2B6	rs8192719	0.94 (0.79, 1.13)	0.51	Dominant	45	39	7	0.291
CYP2B6	rs11882450	1.40 (1.12, 1.76)	0.0042	Dominant	83	8	0	0.044
CYP2B6	rs7255374	1.13 (0.87, 1.47)	0.35	Recessive	41	38	12	0.341
CYP2B6	rs7260329	1.11 (0.91, 1.35)	0.32	Dominant	63	24	4	0.176
CYP2B6	rs3211371	failed quality control						
CYP2B6	rs707265	0.78 (0.64, 0.96)	0.018	Recessive	52	30	9	0.264
CYP2B6	rs1042389	1.48 (1.01, 2.2)	0.046	Recessive	65	22	4	0.165
CYP2B6	rs2113103	1.10 (0.87, 1.38)	0.43	Dominant	78	13	0	0.071
CYP2B6	rs11666982	0.83 (0.67, 1.03)	0.086	Recessive	43	34	12	0.326
CYP2B6	rs7249735	failed quality control						
CYP2B6	rs7255146	1.07 (0.76, 1.51)	0.70	Recessive	53	29	9	0.258
CYP2B6	rs7250597	0.83 (0.65, 1.08)	0.16	Recessive	66	22	3	0.154
CYP2B6	HAP_A8	0.80 (0.63, 1.02)	0.068	Recessive	46	36	9	0.297
CYP2B6	HAP_A10	0.96 (0.80, 1.16)	0.69	Dominant	47	37	7	0.280

CYP2B6	Metabolizer	0.97 (0.85, 1.10)	0.63	Additive	40	37	14	0.357
CYP2C19	rs1998591	0.92 (0.69, 1.22)	0.54	Recessive	35	48	8	0.352
CYP2C19	rs1010570	0.94 (0.73, 1.21)	0.63	Recessive	29	50	12	0.407
CYP2C19	rs12243416	1.09 (0.93, 1.28)	0.26	Additive	54	36	1	0.209
CYP2C19	rs7896133	1.16 (0.97, 1.40)	0.099	Dominant	75	16	0	0.088
CYP2C19	rs1409656	0.97 (0.80, 1.16)	0.71	Dominant	79	12	0	0.066
CYP2C19	rs1409655	failed quality control						
CYP2C19	rs2296684	1.14 (0.87, 1.48)	0.33	Dominant	82	9	0	0.049
CYP2C19	rs2296680	0.85 (0.71, 1.02)	0.081	Dominant	60	28	3	0.187
CYP2C19	rs11188059	1.16 (0.86, 1.55)	0.33	Dominant	85	6	0	0.033
CYP2C19	rs7917985	1.11 (0.95, 1.29)	0.18	Additive	48	39	4	0.258
CYP2C19	rs932809	0.85 (0.71, 1.02)	0.081	Dominant	60	27	4	0.192
CYP2C19	rs10509675	1.08 (0.93, 1.26)	0.32	Additive	54	35	2	0.214
CYP2C19	rs7085563	0.92 (0.81, 1.04)	0.18	Additive	52	33	6	0.247
CYP2C19	rs2281890	0.86 (0.72, 1.03)	0.10	Dominant	57	30	4	0.209
CYP2C19	rs11188067	1.09 (0.93, 1.28)	0.26	Additive	54	36	1	0.209
CYP2C19	rs2860840	0.91 (0.60, 1.40)	0.67	Recessive	69	17	5	0.148
CYP2C19	rs1326830	1.03 (0.81, 1.29)	0.83	Dominant	88	3	0	0.016
CYP2C19	rs7067866	0.89 (0.73, 1.09)	0.27	Recessive	31	43	17	0.423
CYP2C19	rs7101258	1.13 (0.90, 1.43)	0.28	Dominant	80	11	0	0.060
CYP2C19	rs12248560	1.09 (0.93, 1.28)	0.26	Additive	54	36	1	0.209
CYP2C19	rs7916649	0.89 (0.73, 1.09)	0.27	Recessive	31	43	17	0.423
CYP2C19	rs4388808	0.90 (0.68, 1.19)	0.46	Dominant	72	17	2	0.115
CYP2C19	rs4244285	0.83 (0.69, 1.00)	0.051	Dominant	62	26	3	0.176
CYP2C19	rs1322179	0.83 (0.69, 1.00)	0.051	Dominant	62	26	3	0.176
CYP2C19	rs10509677	1.19 (0.90, 1.58)	0.23	Dominant	87	4	0	0.022
CYP2C19	rs7915414	0.95 (0.84, 1.08)	0.43	Additive	44	41	6	0.291
CYP2C19	rs4917623	0.94 (0.82, 1.09)	0.42	Additive	49	34	8	0.275
CYP2C19	rs2104162	0.92 (0.77, 1.11)	0.39	Dominant	35	41	15	0.390
CYP2C19	HAP_A6	0.93 (0.73, 1.20)	0.58	Recessive	42	40	9	0.319
CYP2C19	HAP_A8	1.09 (0.93, 1.28)	0.26	Additive	54	36	1	0.209
CYP2C19	HAP_B8	1.09 (0.93, 1.28)	0.26	Additive	54	36	1	0.209

CYP2D6	rs4147641	0.81 (0.67, 0.99)	0.038	Dominant	27	46	18	0.451
CYP2D6	rs4147638	0.82 (0.68, 0.99)	0.037	Dominant	32	42	16	0.411
CYP2D6	rs3985938	failed quality control						
CYP2D6	rs5758589	1.15 (0.90, 1.47)	0.26	Recessive	39	39	13	0.357
CYP2D6	rs3892097	failed quality control						
CYP2D6	rs9623531	0.86 (0.73, 1.02)	0.077	Dominant	37	45	9	0.346
CYP2D6	rs5758627	1.13 (0.94, 1.35)	0.18	Additive	70	20	1	0.121
CYP2D6	HAP_A1	1.23 (1.01, 1.50)	0.038	Recessive	18	46	27	0.549
CYP2D6	HAP_A3	0.81 (0.67, 0.98)	0.035	Dominant	32	42	17	0.418
CYP2D6	HAP_B1	1.15 (0.90, 1.47)	0.26	Recessive	39	39	13	0.357
CYP2D6	HAP_B2	1.17 (0.88, 1.55)	0.28	Recessive	45	38	8	0.297
CYP2D6	HAP_B3	0.86 (0.73, 1.02)	0.077	Dominant	37	45	9	0.346
CYP3A4A5	rs3735453	1.20 (0.97, 1.49)	0.098	Recessive	41	32	18	0.374
CYP3A4A5	rs11734	0.91 (0.68, 1.22)	0.52	Dominant	79	12	0	0.066
CYP3A4A5	rs7790401	1.08 (0.86, 1.36)	0.49	Additive	78	12	1	0.077
CYP3A4A5	rs10242455	0.87 (0.71, 1.05)	0.14	Dominant	31	37	23	0.456
CYP3A4A5	rs10224569	0.93 (0.78, 1.12)	0.43	Dominant	63	21	7	0.192
CYP3A4A5	rs1419745	1.09 (0.96, 1.24)	0.19	Additive	54	22	15	0.286
CYP3A4A5	rs10264272	0.89 (0.75, 1.05)	0.16	Dominant	74	13	4	0.115
CYP3A4A5	rs4646450	1.13 (0.87, 1.48)	0.35	Recessive	45	29	17	0.346
CYP3A4A5	rs3800959	failed quality control			91	0	0	0.000
CYP3A4A5	rs4646446	1.06 (0.66, 1.71)	0.82	Dominant	86	5	0	0.027
CYP3A4A5	rs10211	1.20 (0.97, 1.48)	0.098	Recessive	40	32	19	0.385
CYP3A4A5	rs2687074	1.09 (0.83, 1.43)	0.52	Recessive	67	17	7	0.170
CYP3A4A5	rs2687144	1.06 (0.93, 1.20)	0.38	Additive	43	34	14	0.341
CYP3A4A5	rs2687136	1.08 (0.89, 1.32)	0.44	Dominant	43	37	11	0.324
CYP3A4A5	rs17161829	1.04 (0.86, 1.25)	0.71	Dominant	59	26	6	0.209
CYP3A4A5	rs12333983	0.96 (0.83, 1.11)	0.56	Additive	29	37	25	0.478
CYP3A4A5	rs17161886	1.11 (0.96, 1.28)	0.17	Additive	49	32	10	0.286
CYP3A4A5	rs4986910	1.99 (1.21, 3.3)			90	1	0	0.005
CYP3A4A5	rs4986909	failed quality control						
CYP3A4A5	rs2242480	failed quality control						

CYP3A4A5	rs28371759	failed quality control			91	0	0	0.000
CYP3A4A5	rs10267228	1.06 (0.90, 1.25)	0.47	Dominant	67	20	4	0.154
CYP3A4A5	rs2687117	0.93 (0.72, 1.21)	0.60	Recessive	58	29	4	0.203
CYP3A4A5	rs4987161	failed quality control						
CYP3A4A5	rs4986907	1.10 (0.91, 1.34)	0.32	Dominant	86	5	0	0.027
CYP3A4A5	rs2738258	1.02 (0.86, 1.22)	0.81	Dominant	46	34	11	0.308
CYP3A4A5	rs7801671	1.16 (0.97, 1.38)	0.10	Dominant	71	18	2	0.121
CYP3A4A5	rs2740574	0.90 (0.73, 1.11)	0.32	Dominant	26	39	26	0.500
CYP3A4A5	rs7811025	1.15 (0.96, 1.39)	0.12	Dominant	75	16	0	0.088
CYP3A4A5	rs493380	failed quality control						
CYP3A4A5	HAP_A2	1.14 (0.87, 1.48)	0.34	Recessive	44	26	17	0.345
CYP3A4A5	HAP_B1	0.96 (0.83, 1.11)	0.56	Additive	29	37	25	0.478
CYP3A4A5	HAP_B2	0.92 (0.76, 1.10)	0.36	Dominant	53	33	5	0.236
CYP3A4A5	HAP_B4	1.04 (0.86, 1.25)	0.71	Dominant	59	26	6	0.209
CYP3A4A5	HAP_C1	0.90 (0.73, 1.11)	0.32	Dominant	26	39	26	0.500
CYP3A4A5	HAP_C2	0.96 (0.84, 1.09)	0.50	Additive	36	41	14	0.379
SLC22A6	rs12223849	1.05 (0.83, 1.34)	0.67	Dominant	66	18	7	0.176
SLC22A6	rs10897310	failed quality control						
SLC22A6	rs2276300	failed quality control						
SLC22A6	rs11568621	failed quality control						
SLC22A6	rs6591722	1.09 (0.91, 1.32)	0.35	Dominant	69	21	1	0.126
SLC22A6	rs11568626	1.07 (0.86, 1.33)	0.56	Dominant	81	10	0	0.055
SLC22A6	rs4149170	0.95 (0.80, 1.13)	0.53	Dominant	54	30	7	0.242
SLC22A6	rs12293966	1.20 (0.96, 1.49)	0.11	Recessive	62	24	5	0.187
SLC22A6	rs955434	0.88 (0.62, 1.25)	0.48	Recessive	66	18	7	0.176
SLC28A2	rs11854484	1.12 (0.95, 1.33)	0.18	Dominant	53	30	8	0.253
UGT1A1	rs7572563	1.05 (0.93, 1.19)	0.39	Additive	47	29	15	0.324
UGT1A1	rs6755571	failed quality control						
UGT1A1	rs7564935	0.98 (0.82, 1.17)	0.82	Dominant	43	36	12	0.330
UGT1A1	rs28900393	1.07 (0.89, 1.28)	0.49	Dominant	76	14	1	0.088
UGT1A1	rs10929302	1.14 (0.81, 1.60)	0.44	Recessive	52	33	6	0.247
UGT1A1	rs3755319	1.07 (0.87, 1.31)	0.52	Dominant	30	42	19	0.440

UGT1A1	rs2003569	0.63 (0.35, 1.11)	0.11	Recessive	55	32	4	0.220
UGT1A1	rs887829	1.07 (0.82, 1.39)	0.63	Recessive	39	42	10	0.341
UGT1A1	rs4148323	0.74 (0.54, 1.01)			89	2	0	0.011
UGT1A1	rs28900396	0.71 (0.45, 1.12)	0.14	Recessive	63	24	4	0.176
UGT1A1	rs28946889	failed quality control						
UGT1A1	rs3771342	1.06 (0.86, 1.29)	0.59	Dominant	66	25	0	0.137
UGT1A1	rs4663971	1.05 (0.85, 1.30)	0.66	Recessive	26	44	21	0.473
UGT1A1	rs929596	0.94 (0.68, 1.29)	0.70	Recessive	49	36	6	0.264
UGT1A1	rs1018124	1.09 (0.91, 1.31)	0.33	Additive	70	20	1	0.121
UGT1A1	rs6431630	failed quality control						
UGT1A1	rs4148328	0.88 (0.74, 1.04)	0.14	Dominant	51	30	10	0.275
UGT1A1	rs11563251	1.20 (0.98, 1.47)	0.078	Recessive	52	29	10	0.269
UGT1A1	rs10929303	1.06 (0.90, 1.26)	0.48	Dominant	44	38	8	0.300
UGT1A1	rs8330	1.05 (0.89, 1.25)	0.55	Dominant	41	43	5	0.298
UGT1A1	rs4148329	0.93 (0.79, 1.10)	0.41	Dominant	32	38	21	0.440
UGT1A1	rs1500482	0.91 (0.81, 1.03)	0.15	Recessive	54	34	3	0.220
UGT1A1	rs4663972	1.05 (0.93, 1.19)	0.41	Additive	36	47	8	0.346
UGT1A1	rs10199882	0.99 (0.81, 1.21)	0.93	Dominant	68	23	0	0.126
UGT1A1	rs9784064	0.99 (0.81, 1.21)	0.92	Dominant	68	23	0	0.126
UGT1A1	rs6746002	0.90 (0.75, 1.09)	0.27	Dominant	63	22	6	0.187
UGT1A1	HAP_A1	1.07 (0.87, 1.31)	0.52	Dominant	30	42	19	0.440
UGT1A1	HAP_A2	0.78 (0.58, 1.06)	0.11	Recessive	44	37	10	0.313
UGT1A1	HAP_A3	1.14 (0.81, 1.60)	0.44	Recessive	52	33	6	0.247
UGT1A1	HAP_B1	0.95 (0.77, 1.18)	0.66	Dominant	21	44	26	0.527
UGT1A1	HAP_B2	1.04 (0.87, 1.25)	0.68	Dominant	53	38	0	0.209
UGT1A1	HAP_B3	0.94 (0.68, 1.29)	0.70	Recessive	49	36	6	0.264
UGT1A1	HAP_C2	1.06 (0.90, 1.26)	0.47	Dominant	44	40	7	0.297
UGT1A1	HAP_C3	0.96 (0.81, 1.13)	0.59	Recessive	9	41	41	0.676
UGT1A1	HAP_D1	0.91 (0.81, 1.03)	0.15	Recessive	55	33	3	0.214
UGT1A1	HAP_D3	1.22 (0.96, 1.55)	0.11	Recessive	55	32	4	0.220
UGT1A1	HAP_D5	0.93 (0.79, 1.10)	0.41	Dominant	32	39	20	0.434

Table 11. Factors associated with 24-hour tenofovir area-under-the-time-concentration-curves exposure in 91 women with HIV.

Factor	**Fold-effect on AUC (± 95% CI)**	**p-value**	**Distribution of factor in sample**
African-American Race	0.95 (0.72, 1.26)	0.7293	55 (60.4%) – no 36 (39.6%) – yes
eGFR < 70 mL/min/1.73 m2	1.30 (0.90, 1.89)	0.1551	82 (90.1%) – no 9 (9.9%) – yes
Principle Component 1	1.09 (1.00, 1.19)	**0.0436**	
Principle Component 2	0.95 (0.90, 1.00)	**0.038**	
Principle Component 3	1.02 (0.97, 1.07)	0.5029	
Age (per decade)	1.14 (1.03, 1.26)	**0.0133**	Median (min, max) – 44.5 (22.9, 64.9)
Body mass index (per 10% increase)	0.95 (0.93, 0.98)	**0.0033**	Median (min, max) – 27 (15, 62)
Ritonavir use	1.26 (1.07, 1.49)	**0.0056**	36 (39.6%) – no 55 (60.4%) – yes
ABCG2 **rs2231142 (one or two rare alleles present as compared to common allele homozygotes)**	1.51 (1.26, 1.81)	**0.000017**	77 (84.6%) – 0 dose 14 (15.4%) – 1 or 2 dose

AUC = area-under-the-time-concentration curve; CI = confidence interval; eGFR = estimated glomerular filtration rate; *ABCG2* = ATP-binding cassette transporter G2

Table 12. Demographic and clinical characteristics of HIV-infected women in biomarker follow-up study at baseline (year 0) and follow-up at year 10.

Parameter	Year 0	Year 10
	N = 294	N = 294
Age (years)	41 (36, 45)	50 (46, 54)
Race		
African-American	176 (60%)	176 (60%)
Caucasian	66 (22%)	66 (22%)
Other	52 (18%)	52 (18%)
Menopausal	50 (17%)	133 (47%)
Cigarette smoking history		
Current	146 (50%)	110 (37%)
Past	81 (28%)	121 (41%)
Never	67 (23%)	63 (21%)
Diagnosis of diabetes mellitus	84 (29%)	84 (29%)
Systolic blood pressure (mm Hg)	120 (110, 129)	119 (109, 133)
Diastolic blood pressure (mm Hg)	72 (68, 80)	74 (67, 82)
Diagnosis of hypertension	71 (24%)	131 (45%)
Antihypertensive use	32 (11%)	104 (35%)
Serum LDL cholesterol (mg/dL)	116 (85, 143)	99 (77, 124)
Serum HDL cholesterol (mg/dL)	45 (36, 55)	53 (41, 64)
Serum triglyceride (mg/dL)	126 (87, 174)	110 (86, 156)
Body mass index (kg/m2)	27 (23, 32)	28 (24, 33)
Waist circumference (centimeters)	88 (80, 100)	94 (85, 107)
Current HAART use	186 (64%)	264 (90%)
History of AIDS	149 (51%)	181 (62%)
Hepatitis C virus infection	79 (27%)	79 (27%)
Current CD4 cell count (cells/microliter)	394 (269, 587)	520 (326, 698)
Lifetime nadir CD4 cell count (cells/microliter)	240 (133, 337)	163 (79, 258)
Current HIV RNA < 80 copies/mL	95 (32%)	196 (67%)
Lifetime peak HIV RNA > 10,000 copies/mL	221 (75%)	255 (87%)
eGFRcr (mL/min/1.73 m2)	97 (82, 112)	95 (76, 109)
Data are presented as Median (IQR) or numbers (percent).		

Abbreviations: IQR, interquartile range; eGFRcr, estimated glomerular filtration rate by serum creatinine; HAART, highly active antiretroviral therapy

Table 13. Characteristics associated with change from baseline to year 10 in urine biomarker levels using multivariable-adjusted MANOVA.

Variable	IL-18 % Estimate (95%CI)	α1m % Estimate (95%CI)	ACR % Estimate (95%CI)
Parsimonious model, non-significant factors dropped			
Y0 body mass index (kg/m2)	**2.5 (1.48, 3.5) p<.0001**		
Change from baseline body mass index (kg/m2)		**-2.1 (-3.6, -0.55) p=0.0079**	
Change from baseline waist circumference (per 10 cm)	**15.3 (7.8, 23.2) p<.0001**		
Y10 HDL (per 10 mg/dL)	**-5.6 (-9.2, -1.81) p=0.0040**		
Y10 triglycerides (per doubling)			**11.5 (0.90, 23.1) p=0.033**
Y0 smoking		**22.3 (5.2, 42.1) p=0.0086**	
Y0 diastolic blood pressure (per 10 mm Hg)		**7.3 (0.80, 14.2) p=0.027**	
Y10 diastolic blood pressure (per 10 mm Hg)			**9.0 (1.95, 16.6) p=0.012**
Hepatitis C virus infection	**27.3 (9.7, 47.7) p=0.0015**	**25.6 (6.4, 48.4) p=0.0072**	**22.7 (4.1, 44.7) p=0.015**
Nadir CD4 prior to baseline (per doubling)		**9.5 (3.8, 15.6) p=0.00097**	
Nadir CD4 between Y0 and Y10 (per doubling)		**-10.1 (-14.2, -5.7) p<.0001**	
Y10 CD4 Count (per doubling)	**-10.7 (-16.5, -4.5) p=0.0010**		
Y10 HIV RNA (per 10 fold increase)	**19.2 (10.1, 29.0) p<.0001**		**9.7 (1.37, 18.7) p=0.022**
Adjusted R2	0.33	0.23	0.18

Abbreviations: IL-18, urine interleukin 18; α1m, urine alpha-1-microglobulin; ACR, urine albumin:creatinine ratio; Y0, baseline year 0; Y10, year 10 follow-up

Table 14. Change from baseline to year 10 in urine biomarker levels using multivariable-adjusted MANOVA (full model).

Variable	IL-18 % Estimate (95%CI)	α1m % Estimate (95%CI)	ACR % Estimate (95%CI)
Full model, all factors forced for each outcome			
Y0 body mass index (kg/m2)	**2.5 (1.38, 3.5)** **p<.0001**	-0.83 (-1.94, 0.28) p=0.14	-0.25 (-1.41, 0.92) p=0.67
Change from baseline body mass index (kg/m2)	-1.17 (-3.5, 1.27) p=0.34	**-3.0 (-5.6, -0.45)** **p=0.022**	0.60 (-2.1, 3.4) p=0.67
Change from baseline waist circumference (per 10 cm)	**18.6 (6.5, 32.1)** **p=0.0019**	4.6 (-6.8, 17.4) p=0.44	0.74 (-10.7, 13.7) p=0.90
Y10 HDL (per 10 mg/dL)	**-6.7 (-10.6, -2.7)** **p=0.0013**	1.71 (-2.8, 6.4) p=0.46	-0.01 (-4.7, 4.9) p=0.99
Y10 triglycerides (per doubling)	-8.8 (-17.6, 0.80) p=0.071	9.6 (-1.79, 22.2) p=0.10	11.2 (-0.98, 24.8) p=0.073
Y0 smoking	**18.7 (2.8, 37.2)** **p=0.020**	**18.3 (1.61, 37.6)** **p=0.030**	0.81 (-14.1, 18.2) p=0.92
Y0 diastolic blood pressure (per 10 mmHg)	-1.39 (-7.7, 5.4) p=0.68	6.3 (-0.99, 14.1) p=0.092	-1.81 (-8.9, 5.8) p=0.63
Y10 diastolic blood pressure (per 10 mmHg)	4.7 (-1.83, 11.6) p=0.16	4.2 (-2.7, 11.6) p=0.24	**10.0 (2.2, 18.3)** **p=0.011**
Hepatitis C virus infection	**18.0 (0.63, 38.4)** **p=0.042**	**22.7 (3.4, 45.6)** **p=0.019**	**23.2 (2.9, 47.5)** **p=0.023**
Nadir CD4 prior to baseline (per doubling)	0.37 (-4.5, 5.5) p=0.88	**8.8 (3.0, 14.9)** **p=0.0025**	-1.01 (-6.4, 4.7) p=0.72
Nadir CD4 between Y0 and Y10 (per doubling)	-0.80 (-6.1, 4.8) p=0.78	**-8.1 (-13.3, -2.6)** **p=0.0045**	1.75 (-4.3, 8.2) p=0.58
Y10 CD4 Count (per doubling)	**-8.3 (-15.6, -0.33)** **p=0.042**	-3.9 (-12.1, 5.0) p=0.38	-0.39 (-9.3, 9.4) p=0.93
Y10 HIV RNA (per 10 fold increase)	**18.0 (8.9, 27.9)** **p<.0001**	2.3 (-6.2, 11.5) p=0.61	**10.2 (0.56, 20.7)** **p=0.038**

Abbreviations: IL-18, urine interleukin 18; α1m, urine alpha-1-microglobulin; ACR, urine albumin:creatinine ratio; Y0, baseline year 0; Y10, year 10 follow-up

Table 15. Cluster membership analysis using multivariable adjusted multinomial logistic regression.

Parameter	Cluster 2 vs. Cluster 1 Odds Ratio (95%CI)	Cluster 3 vs. Cluster 1 Odds Ratio (95%CI)	Cluster 4 vs. Cluster 1 Odds Ratio (95%CI)
Current smoker	4.55 (2.19, 9.44) p<.0001	2.23 (1.07, 4.66) p=0.033	1.13 (0.58, 2.19) p=0.72
Time-averaged CD4 (per doubling)	0.51 (0.31, 0.82) p=0.0059	0.77 (0.46, 1.30) p=0.33	0.56 (0.35, 0.89) p=0.013
History of AIDS	2.71 (1.33, 5.51) p=0.0061	1.78 (0.85, 3.71) p=0.12	1.67 (0.87, 3.20) p=0.12

Notes: Time-averaged CD4 count over years 0 – 10.