Pharmacogenetics of Tenofovir (Alafenamide or Disoproxil Fumarate Prodrug) Renal Toxicity in HIV-positive Black Southern Africans

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Declaration

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Manuscript

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Somila Mateza was responsible for the selecting candidate genes, data cleaning (using STATA), data merging (genetic and clinical data using PLINK), analysis (running linear regression analysis), data interpretation, and drafted the manuscript, which was critically reviewed by all authors. Yuki Bradford contributed to the post-genotyping quality control, imputation and the genetic association analyses. Gary Maartens contributed to the study design, data interpretation and critically reviewed the manuscript. Simiso Sokhela, Nomathemba C. Chandiwana and Willem D. F. Venter are the investigators on the parent study, ADVANCE clinical trial, and they contributed to the acquisition of clinical data and critically reviewed the manuscript. Frank A. Post contributed to the study design, data interpretation and critically reviewed the manuscript. Marylyn D. Ritchie contributed to imputation and genetic association analyses and reviewed the manuscript. David W. Haas contributed to study design, supervised genotyping at VANGARD and the genetic association analyses and interpretation and critically reviewed the manuscript. Phumla Sinxadi is the Principal Investigator of the study and she contributed to the study design, data interpretation, and critically reviewed the manuscript.

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Abbreviations

%CV - Percentage of coefficient of variation

3TC - Lamivudine

ADVANCE - Phase 3 Non-inferiority Study of DTG + TAF + FTC Compared with DTG + TDF + FTC and EFV + TDF + FTC in Patients Infected with HIV-1 Starting First-line Antiretroviral Therapy

ART - Antiretroviral Therapy

AUC - Area under the curve

B2M - Beta2-Microglobulin

BMI - Body mass index

CD4 - Cluster of Differentiation 4

CKD - Chronic kidney disease

CKP-Epi - Chronic Kidney Disease Epidemiology Collaboration equation

C_{max} - Maximum drug concentration

C_{min} - Trough drug concentration

CrCl - Creatinine clearance

DTG - Dolutegravir

eGFR - Estimated Glomerular Filtration Rate

GWAS - genome-wide association study

HIV - Human Immunodeficiency Virus

HWE - Hardy-Weinberg equilibrium

IQR - Interquartile range

IRR - Incidence rate ratio

JAC - the Journal of Antimicrobial Chemotherapy

MAF - Minor allele frequency

MRP - Multidrug resistant protein

NRTI - Nucleoside Reverse-Transcriptase Inhibitor

OAT - Organic anion transporters

OR - Odds ratio

P - P-value

PBMCs - peripheral blood mononuclear cells

QC - Quality control

RBP - Retinol Binding Protein

RNA - Ribonucleic Acid

SNP - Single Nucleotide Polymorphism

$T_{1/2}$ - Half life

TAF - Tenofovir Alafenamide Fumarate

TDF - Tenofovir Disoproxil Fumarate

TFV - Tenofovir

TFV-DP - Tenofovir Diphosphate

uB2M/Cr - Urine beta-2-microglobulin adjusted for urinary creatinine

UNAIDS - The Joint United Nations Programme on HIV/AIDS

uRBP/Cr - Urine retinol binding protein adjusted for urinary creatinine

WHO - World Health Organisation

ΔB2M:Cr - Change in urine beta-2-microglobulin adjusted for urinary creatinine

ΔeGFR - Change in estimated Glomerular Filtration Rate

ΔRBP:Cr - Change in urine retinol binding protein adjusted for urinary creatinine

Abstract

Background: Among individuals treated for HIV-1 infection, renal toxicity is more likely with tenofovir disoproxil fumarate (TDF) than with tenofovir alafenamide (TAF). Limited previous studies suggest potential genetic associations with TDF-associated renal toxicity. We hypothesized that polymorphisms in genes of potential relevance to tenofovir, TDF and TAF disposition are associated with renal toxicity in people living with HIV in Southern Africa.

Material and Methods: Adult participants randomized to initiate TAF or TDF (each given with emtricitabine) in the dolutegravir arms of the ADVANCE trial had the option to co-enrol in a pharmacogenetic sub-study. We assessed changes from week 4 (to minimize impact of early dolutegravir-induced increases in creatinine) to week 48 in estimated glomerular filtration rate (eGFR-CKD-EPI), and log-transformed changes from baseline to week 48 in urine retinol binding protein and urine β2-microglobulin, each adjusted for urinary creatinine (uRBP/Cr and uB2M/Cr, respectively). Genotyping was done using Illumina MEGAEX, followed by imputation with TOPMed. Genetic associations with each renal outcome (eGFR, uRBP/Cr and uB2M/Cr) were assessed using multivariable linear regression models adjusting for age, sex, treatment group, and screening body mass index, CD4 count, and $\log_{10}$ HIV-1 RNA. Primary analyses prioritized 14 polymorphisms previously reported to be associated with tenofovir disposition or renal tubular dysfunction, and all polymorphisms (+/- 50 kB) in selected genes of interest: *ABCB1*, *ABCC2*, *ABCC4*, *ABCC10*, *ABCG2*, *AK2*, *AK3*, *CES1*, *CYP3A4*, *NME1*, *SLC22A2*, *SLC22A6*, *SLC22A8* and *SLC22A11*. We also explored associations genome-wide.

Results: In ADVANCE, 336 participants randomized to either TAF or TDF consented to genetic testing. All were Black-African, 63% were female, median age was 32 years, CD4 count 292 cells/μL, and $\log_{10}$ HIV-1 RNA 4.4 copies/mL. Among the 14 polymorphisms of primary interest, the lowest P-values for change in eGFR, uRBP/Cr and uB2M/Cr were *ABCC4* rs899494 (P = 0.021), *ABCC10* rs2125739 (P = 0.07), and *ABCC4* rs1059751 (P = 0.0087), respectively. Among genes of interest, the lowest P-values for change in eGFR, uRBP/Cr and uB2M/Cr were *ABCC4* rs4148481 (P = 1.5×10^{-4}), rs691857 (P = 3.2×10^{-4}), and *PKD2* rs72659631 (P = 8.6×10^{-4}), respectively. In genome-wide analyses,

the lowest P-values for change in eGFR, uRBP/Cr, and uB2M/Cr were *COL27A1* rs1687402 (P = 3.2×10^{-9}), *CDH4* rs66494466 (P = 3.4×10^{-8}), and *ITGA4* rs3770126 (P = 4.5×10^{-7}), respectively.

Conclusions: Among Southern African participants in a randomized trial of dolutegravir plus either TAF/emtricitabine or TDF/emtricitabine, two polymorphisms previously associated with TDF renal toxicity, *ABCC4* rs899494 and *ABCC4* rs1059751, were nominally associated with change in eGFR and uB2M/Cr, respectively, but did not withstand correction for multiple testing (nor did associations in genes of interest). A polymorphism in *COL27A1*, which encodes collagen type XXVII alpha 1 chain, was genome-wide associated with change in eGFR. These findings enhance our understanding of the impact of human genetics on tenofovir-associated renal toxicity.

Introduction

The estimated number of people living with HIV in South Africa is 7.8 million, the largest HIV epidemic in the world and 72% of these individuals are receiving antiretroviral therapy (ART).[1] The standardised national ART regimens for adults and adolescents in South Africa is tenofovir disoproxil fumarate (TDF) + Lamivudine (3TC) + Dolutegravir (DTG), [2] which follows the 2019 World Health Organisation (WHO) recommendation of a combination of dolutegravir with a nucleoside reverse-transcriptase inhibitor (NRTI) backbone.[3]

In 2015 the WHO recommended that all adolescents and adults living with HIV at any CD4 cell count be started on ART.[4] This recommendation has had significant programmatic and financial implications especially in low- and middle-income countries like South Africa.[5] The Treat All recommendation is recognized as the primary strategy in the goal of achieving the UNAIDS's 90-90-90 targets and most sub-Saharan Africa countries had adopted the recommendation by 2017.[5,6] This has resulted in an increased focus on optimising treatment to a more effective, cheaper and less harmful treatment.[7]

Tenofovir disoproxil fumarate, a prodrug of tenofovir (TFV), is recommended by the WHO as part of first-line ART regimens.[3] But in some high income countries there has been a push towards tenofovir alafenamide (TAF) because it has a more favourable toxicity profile and a smaller tablet size while remaining equally effective.[7–10] TDF use is now an established risk factor for renal toxicity.[11] It has been associated with decreased creatine clearance,[12] acute kidney injury and tubular dysfunction.[12] The severity of renal toxicity with TDF correlates with higher plasma tenofovir concentrations.[13] TAF is associated with lower plasma tenofovir concentrations and increased intracellular concentrations of tenofovir diphosphate (TFV-DP) compared to TDF.[14]

Genetic variants in genes involved in transport and metabolism of TDF, TAF and tenofovir have been postulated to increase risk of renal toxicity by favouring intracellular accumulation of tenofovir. Candidate gene studies from various populations have sought to associate renal transporter polymorphisms with TDF renal toxicities among individuals treated for HIV, most of which did not include African populations. Because of inconsistent results from pharmacogenomic studies and the

limited data regarding the pharmacogenetics of TDF toxicity among Africans, who have the highest genetic diversity worldwide, we evaluated whether laboratory markers of renal tubular injury were associated with polymorphisms in genes involved in TAF, TDF and tenofovir metabolism and transport among ART-naïve Southern Africans who had been randomized to initiate either TDF- or TAF-containing regimens in a prospective clinical trial.

My study utilises data from the two dolutegravir arms of the ADVANCE randomised clinical trial (NCT03122262). The thesis will comprise the narrative literature review and the manuscript submitted to the *Journal of Antimicrobial Chemotherapy* (JAC), and appendices (JAC author guidelines, ethics approval letter and protocol).

Literature review

Tenofovir prodrugs

Tenofovir disoproxil fumarate is a once-daily nucleotide analogue reverse transcriptase inhibitor with activity against HIV-1 and HIV-2, and is the first NRTI to be approved for HIV treatment.[15] TDF is a disoproxil diester prodrug of tenofovir, the diester moieties increase the lipophilicity of tenofovir and thus its permeability.[15] The metabolism of TDF is outlined in **Figure 1**.[16]

After multiple administrations of TDF once daily 300 mg dose with a meal in HIV positive adults, a mean tenofovir Cmax = 0.33 mg/L is observed which is reached within 2 hours and within 1 hour in fasted state, which is consistent with delaying effects of food on gastric emptying.[17] The Cmin = 0.06 mg/L (percentage of coefficient of variation (%CV) = 39.4%) and AUC = 3.32 mg.h/L (%CV = 41.2%). The oral bioavailability is approximately 25% without food and increases to 40% with a high fat meal but a light meal did not have a significant effect on the pharmacokinetics of TDF.[17,16] Intravenous administration of TDF 1 mg/kg has a mean steady-state volume of distribution of

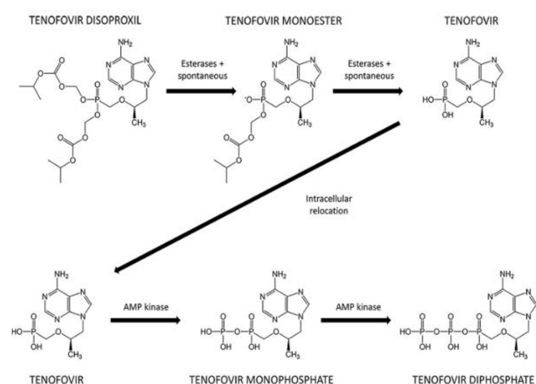

Figure 1: Conversion of tenofovir disoproxil fumarate to tenofovir and ultimately to the active substance tenofovir-diphosphate. *Upon oral administration, TDF undergoes esterase hydrolysis mediated by carboxylesterase enzymes, which removes the two ester groups. Cleavage of the first ester group yields the monoester intermediate, while cleavage of the second ester group yields*

approximately 800 ml/kg indicating that the volume of distribution approximates the total body water volume.[18] Tenofovir alafenamide (TAF) is a novel prodrug of tenofovir with improved renal toxicity compared to TDF.[19,20] TAF is hydrolysed by cathepsin A in peripheral blood mononuclear cells and is primarily hydrolysed to form tenofovir by carboxylesterase 1 in primary hepatocytes.[20,21] This step releases TFV-alanine that is further hydrolysed to TFV. Tenofovir alafenamide is mainly eliminated following metabolism to tenofovir, only <1% of intact TAF is eliminated in urine.[22] After oral administration of 25mg TAF a mean tenofovir Cmax = 15.7 ng/mL was observed at fasted state and the AUC = 267.7 ng.h/mL. [14]

Table 1: Steady state pharmacokinetics of TFV through 10 days of monotherapy (TAF and TDF).[14]

Pharmacokinetic Parameters	**25 mg TAF**	**300 mg TDF**
AUC_{tau} (ng.h/ml), mean (%CV)	267.7 (26.7)	1918.0 (39.4)
C_{max} (ng/ml), mean (%CV)	15.7 (22.1)	252.1 (36.6)
C_{tau} (ng/ml), mean (%CV)	9.2 (26.1)	38.7 (44.7)
T_{max} (h), median (Q1, Q3)	1.50 (1.25, 1.75)	1.25 (0.58, 2.00)
$T_{1/2}$ (h), median (Q1, Q3)	40.19 (28.9, 44.8)	14.86 (12.2, 16.8)

Compared to TDF, TAF is more stable in plasma and is predominantly converted to TFV intracellularly.[14] TAF is not a substrate of organic anion transporters (OAT)1 and 3 therefore this reduces the exposure of TFV in proximal tubule cells and may lead to less nephrotoxicity.[14,23] Monotherapy with TAF 25 mg for 10 days resulted in 86% lower mean TFV AUC_{tau} than 300 mg TDF.[14] TAF produces higher concentrations of the active metabolite tenofovir diphosphate (TFV-DP). In peripheral blood mononuclear cells (PBMCs), a 7-fold increase was reported when participants were

dosed with 25 mg TAF compared to the standard dose of TDF.[14] Studies have shown that TFV-related toxicity is linked to higher TFV plasma concentrations.[13] Therefore, TAF is more favourable than TDF because it produces 90% less plasma TFV.[10]

Tenofovir, derived from either TDF or TAF, is then phosphorylated intracellularly by the cellular nucleotide kinase, adenylate kinase, to the monophosphate intermediate, and then rapidly converted by nucleoside diphosphate kinase to TFV-DP.[15] Tenofovir is primarily excreted by the kidney by both passive glomerular filtration and active tubular secretion. Tenofovir is actively transported into proximal renal tubular cells by OAT1 and OAT3, which are encoded by *SLC22A6* and *SLC22A8*, respectively. It is then transported out of these cells by efflux transporters including multidrug resistant protein 4 (MRP4, encoded by *ABCC4*).[24]

Tenofovir Associated Renal Toxicity

Although usually safe and well tolerated, TDF use is now an established risk factor for renal toxicity.[11] It has been associated with decreased creatine clearance,[12] acute kidney injury and tubular dysfunction.[12] TDF-induced nephrotoxicity is associated with higher concentrations of TFV in proximal tubule cells which may be resulting from high plasma concentrations of TFV when given as TDF.[13,25,]

In a French cohort (A cross-sectional survey was carried out within the ANRS C03 Aquitaine cohort) TDF was significantly associated with proximal renal tubular dysfunction (OR = 1.23 per cumulative year of exposure, P=0.028) adjusting for age, non-steroidal anti-inflammatory drugs and other antiviral drugs exposure, chronic hepatitis B, lipodystrophy, and cumulated exposure to abacavir, atazanavir, and ritonavir used as boosters. Proximal renal tubular dysfunction remained associated with TDF in participants who remained on TDF-containing regimens over 5 years with OR of 5.22.[26]

A study from the D:A:D study (participants in Europe, the United States and Australia) aimed to investigate associations between exposure to antiretrovirals and chronic kidney disease (CKD) in participants with normal eGFR (normal eGFR was defined as > 90 mL/min/1.73m^2) at baseline. They found that cumulative exposure to TDF was significantly associated with increased incidence of CKD. Specifically, with each additional year of exposure to TDF, adjusted incidence rate ratio (IRR) of

developing CKD was increased by 14% [adjusted IRR 1.14; (95% CI 1.10 to 1.19)].[27] These findings were also reported in a Canadian study that reported cumulative incidence of reduced kidney function. In this study they showed that after 2, 5, and 10 years of exposure to TDF, the reduced kidney function was 15.12% (95% CI, 11.15 to 20.34), 31.47% (95% CI, 26.17 to 37.54), and 52.29% (95% CI, 45.65 to 59.26), respectively. When compared to other classes of ART, tenofovir exposure increased the risk of reduced kidney function by 63%.[28]

Studies of tenofovir toxicity in African populations have yielded inconsistent results.[29] Some found tenofovir-containing regimens to be without substantial renal toxicity,[30, 31] while others found increased renal toxicity and recommend monitoring of renal function.[32–34] These studies had varying study designs and sample sizes, which could explain the inconsistent results. A cross sectional study of 953 HIV-infected Ugandan participants aimed to compare the renal function of participants on TDF with participants on non-TDF treatment regimen. Proportion of renal dysfunction was slightly higher in participants on TDF, but the difference was not statistically significant. Hyperphosphatemia was the only renal tubular marker that was higher in the non-TDF treatment group. A multivariable analysis adjusting for age, sex, BMI, duration on the current ART regimen, alcohol consumption, viral load and CD4 cell counts found no significant differences in renal function between the TDF and non-TDF groups, and they concluded that tenofovir based first line ART can be safely initiated even in settings without routine renal function monitoring.[30]

A cross sectional study of 166 HIV-infected Cameroonian participants aimed to determine the prevalence of TDF-induced renal dysfunction in participants that were treatment experienced (n = 119) versus participants that were treatment naïve (n = 47). Treatment experienced participants had higher eGFR values compared to treatment naïve participants with 78.2 mL/min and 71.4 mL/min respectively. They concluded that TDF seems to be safe and does not appear to be a significant cause of renal impairment in their cohort.[31]

A cross sectional study of 445 HIV-infected Zambian participants, investigated the proportions of participants developing TDF-induced renal dysfunction. Disease was defined by CrCl ≤ 50 ml/min, ART combinations were TDF/emtricitabine/nevirapine or TDF/emtricitabine/efavirenz. After 18

months of follow up the overall prevalence of renal dysfunction was 18.6%, 43 participants had CrCl < 50 ml/min.[32]

In a South African retrospective cohort study of 650 HIV-infected participants initiating TDF. Participants experience a median decline in eGFR of -3.53 mL/min/1.73m^2 over time and over half of the participants (361) developed rapid kidney function decline and 15 (2%) progressed to stage 3 CKD during a median follow-up of approximately 30 and 54 weeks, respectively.[33]

In another South African cohort study of more than 15000 patients, the mean CKD-Epi eGFR change from baseline to 12 months was −1.0 mL/min (−1.2 to −0.8) but varied by baseline renal function. In patients with baseline CKD-Epi eGFR ≥90 mL/min the predicted mean change in eGFR from baseline to 12 months was −2.3 mL/min (−2.5 to −2.1) and 14.6 (13.5 to 15.7) in patients with baseline eGFR <90 mL/min.[34] In both this study and the previous participants with moderate to severe kidney impairment (eGFR <90 mL/min) eGFR improved upon initiating TDF-containing treatment. This improvement is speculated to be due to effective treatment of underlying illnesses present at ART initiation. The summary of the studies on TDF associated renal toxicity is displayed in Table 2.

Table 2: Studies previously investigating TDF related renal toxicity.

Study and Country	**Sample size**	**Outcome**	**Main findings/comments**
Prospective or retrospective cohort studies			
Mocroft et al,[27] Multicentre (Europe, United States, Australia)	23 905	Chronic kidney disease (defined as confirmed (>3 months apart) eGFR lower than 70 mL/min/1.73 m²)	The effect of the association between TDF, ritonavir-boosted atazanavir, or ritonavir-boosted lopinavir was cumulative and after 5 years of exposure the incidence of chronic kidney disease increased by two-to-three-fold.

Laprise et al,[28] Canada	1043	Kidney dysfunction (defined as eGFR <90 mL/min/1.73 m^2)	When compared to other classes of ART, tenofovir exposure increased the risk of renal dysfunction.
Zachor et al,[33] South Africa	650	Stage 3 chronic kidney disease (defined as eGFR <60 ml/min/1.73 m^2)	Median decline in eGFR of -3.53 mL/min/1.73m^2 over time and over half of the participants (361) developed rapid kidney function decline and 15 developed stage 3 CKD.
De Waal et al,[34] South Africa	>15000	Estimated glomerular filtration rate (normal eGFR defined as ≥90 mL/min)	Patients on TDF with normal baseline eGFR experienced small but significant declines in eGFR. However, eGFR improved in patients with baseline eGFR <90 mL/min, regardless of which estimating equation was used.
Cross-sectional studies			
Salome et al,[30] Uganda	953	Renal function (defined by measured renal function tests, and calculated Fractional Tubular reabsorption of phosphate and eGFR)	They found no significant differences in renal function between the TDF and non-TDF groups.
Fritzsche et al,[31] Cameroon	166	Renal dysfunction (defined as eGFR < 60 mL/min)	Treatment with TDF was not associated with an increase in serum creatinine or a decrease in eGFR after 6 months of therapy.

Wantakischa et al,[32] Zambia	445	Renal dysfunction (defined as CrCl level ≤ 50 ml/min)	The study showed a higher prevalence (18.6%) of renal dysfunction among HIV positive adults on TDF-based therapy.
Dauchy et al,[26] France	399	Proximal renal tubular dysfunction (defined by the presence of two or more of the following parameters: glucosuria, hypophosphatemia, metabolic acidosis, hypouricemia, and proteinuria)	The study showed a significant association between proximal renal tubular dysfunction and TDF exposure in univariable and multivariable analysis.

Genetic studies

Tenofovir is primarily excreted by the kidney by both passive glomerular filtration and active tubular secretion. Tenofovir is actively transported into proximal renal tubular cells by organic anion transporters 1 and 3 (OAT1 and OAT3, encoded by *SLC22A6* and *SLC22A8*, respectively), and transported out of these cells by efflux transporters including multidrug resistant protein 2 and 4 (MRP2 and MRP4, encoded by *ABCC2* and *ABCC4*).[24] Genetic variations of the transporter proteins have been identified to be linked with increased serum concentrations of TFV.[25]

A host of candidate gene studies from various populations have sought to identify associations of renal transporter polymorphisms and TDF renal toxicities among individuals being treated for HIV, most of which did not include African populations.

A study of 30 predominantly white French participants, reported an association between the polymorphism 1249 G→A (rs2273697) in *ABCC2* and TDF-induced renal proximal tubulopathy,

defined by a standard screening protocol in line with Gilead treatment-surveillance recommendations.[35] Thirteen developed renal proximal tubulopathy and 17 participants did not.

In a cross-sectional study of 115 Spanish participants, 12 polymorphisms in the *ABCC2*, *ABCC4*, *SCL22A6*, *SLC22A11*, and *ABCB1* genes were analysed for association with kidney tubular dysfunction. Disease was defined by the presence of at least 2 of the following abnormalities: nondiabetic glucosuria, urine phosphate wasting, hyperaminoaciduria, β2-microglobulinuria, and increased fractional excretion of uric acid. The genotype CC at *ABCC2* -24 (rs717620) was associated with kidney tubular dysfunction.[36] On the same study population with the same criteria used to diagnosis kidney tubular dysfunction, 14 polymorphisms in *ABCC10* were selected and screened. Two polymorphisms, rs9349256 and rs2125739, were associated with kidney tubular dysfunction.[37] A study of 190 Japanese patients found an association between TDF-induced renal tubular dysfunction and 2 *ABCC2* polymorphisms (rs717620 and rs2273697).[38] Disease was defined by the presence of at least 3 of the following abnormalities: fractional tubular resorption of phosphate, fractional excretion of uric acid, urinary β2-microglobulin, urinary α1-microglobulin, and urinary N-acetyl-β-D-glucosaminidase.

In a genome wide association study among patients randomised to receive TDF/emtricitabine in the ACTG A5202 study, no significant genome wide associations with change in creatinine clearance (CrCl) were found. However, in candidate single nucleotide polymorphism (SNP) analyses stratified by population, *SLC22A2* rs3127573 was significantly associated with a positive 6 month change among African Americans.[39]

In light of scant data regarding the pharmacogenetics of TDF nephrotoxicity among Africans, it is important to investigate these populations. A study of 66 participants from Ghana, investigated SNPs in *ABCC10*, *ABCC2* and *ABCC4* for association with tenofovir renal clearance, kidney tubular dysfunction, chronic kidney disease and individual biochemical parameters (Creatinine clearance, serum, and urine creatinine concentrations). They reported an association of *ABCC10* 2843T>C (rs2125739) with indicators of declining renal function (i.e. lower creatinine clearance at time of sampling).[40]

African populations have greater genetic diversity compared with Europeans.[41,42] Considering that Southern Africa has the largest number of people living with HIV and individuals of African ancestry have a higher risk of HIV associated nephropathy.[43,44] It is important to investigate this group.

Considering this, the present study was designed to characterize whether several laboratory markers of renal tubular function were associated with polymorphisms in genes involved in TAF, TDF and/or tenofovir metabolism and transport and among ART-naïve Southern Africans who had been randomized to receive either TDF- or TAF-containing regimens in a prospective clinical trial.

Summary of literature review

At a standard dose of 300 mg, TDF is normally well tolerated. However, it has been associated with renal toxicity, which is associated with high plasma concentrations. With its widespread use and increase in life expectancy for people living with HIV, it is increasingly important to understand adverse effects related to TDF. TAF is a newer prodrug of TFV with a more improved toxicity profile and a lower recommended daily dose (25 mg). Studies have shown that TFV-related toxicity is linked to higher TFV plasma concentrations. Therefore, TAF is more favourable than TDF because it produces 90% less plasma TFV. Candidate gene studies have suggested associations between polymorphisms and adverse renal effects with tenofovir-containing regimens. Many of the gene studies though did not include participants of African descent, but novel polymorphisms are more likely to be found in African populations because of their high genetic diversity. There are contradicting reports on renal safety of TDF and limited clinical evidence on the efficacy and safety of TAF in low to middle income country populations in general.

Knowledge gaps identified

No previous study evaluated polymorphisms associated with TFV change in renal markers among participants randomized to TAF. We evaluated whether laboratory markers of renal tubular injury were associated with polymorphisms in genes involved in TAF, TDF and tenofovir metabolism and transport among ART-naïve Southern Africans who had been randomized to initiate either TDF- or TAF-containing regimens.

Hypothesis

We hypothesized that some selected and novel polymorphisms in candidate genes involved in metabolism and transport of TAF or TDF will be associated with change in markers of renal toxicity in our population.

Aim

To determine if known polymorphisms and novel polymorphisms in the candidate genes *ABCB1*, *ABCC10*, *ABCC2*, *ABCC4*, *ABCG2*, *AK2*, *AK3*, *CES1*, *CYP3A4*, *NME1*, *SLC22A6*, *SLC22A8* and *SLC22A11*, will be associated with tenofovir related change in renal markers of toxicity in an HIV-positive Southern African population.

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Manuscript

Pharmacogenetics of Tenofovir Renal Toxicity in HIV-positive Southern Africans

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Synopsis

Introduction: Renal toxicity is more likely with tenofovir disoproxil fumarate (TDF) than with tenofovir alafenamide (TAF). We hypothesized that polymorphisms in genes relevant to tenofovir, TDF, and TAF disposition affect renal toxicity risk among HIV-positive Southern Africans.

Methods: Adults randomized to initiate TAF or TDF in the dolutegravir arms of the ADVANCE trial were studied. Outcomes were change from week 4 to 48 in estimated glomerular filtration rate (eGFR), and from baseline to week 48 in urine retinol binding protein and urine β_2-microglobulin, adjusted for urinary creatinine (uRBP/Cr and uB2M/Cr). Associations were assessed using multivariable linear regression models. Primary analyses prioritized 14 polymorphisms previously reported to be associated with tenofovir disposition or renal outcomes, and all polymorphisms in 14 selected genes. We also explored genome-wide associations.

Results: Analyses included 336 ADVANCE participants. Among 14 polymorphisms of primary interest, the lowest P-values for change in eGFR, uRBP/Cr and uB2M/Cr were *ABCC4* rs899494 (P=0.021), *ABCC10* rs2125739 (P=0.07), and *ABCC4* rs1059751 (P=0.0087), respectively. In genes of interest, the lowest P-values were *ABCC4* rs4148481 ($P=1.5x10^{-4}$), rs691857 ($P=3.2x10^{-4}$), and *PKD2* rs72659631 ($P=8.6x10^{-4}$), respectively. Genome-wide, the lowest P-values were *COL27A1* rs1687402 ($P=3.2x10^{-9}$), *CDH4* rs66494466 ($P=3.4x10^{-8}$), and *ITGA4* rs3770126 ($P=4.5x10^{-7}$), respectively.

Conclusions: Two polymorphisms previously associated with TDF renal toxicity, *ABCC4* rs899494 and *ABCC4* rs1059751, were nominally associated with change in eGFR and uB2M/Cr, respectively, albeit in the opposite direction of previous reports. A *COL27A1* polymorphism was genome-wide significant and associated with change in eGFR. These findings enhance our understanding of the genetics of tenofovir-associated renal toxicity.

Introduction

Tenofovir disoproxil fumarate (TDF), a prodrug of tenofovir, is recommended by the World Health Organization as part of first-line antiretroviral therapy (ART) regimens.[1] In 2015, the United States Food and Drug Administration approved a second tenofovir prodrug, tenofovir alafenamide fumarate (TAF), for inclusion in ART regimens.[2] Compared to TDF, TAF is as effective but has a more favourable safety profile.[3–5] The prescribing shift from TDF to TAF has been driven, in part, to avoid TDF renal tubular toxicity.[6–10] The severity of renal toxicity with TDF correlates with higher plasma tenofovir concentrations.[11] Plasma tenofovir concentrations with TAF are approximately 90% lower than those with TDF.[3, 5]

TDF undergoes esterase hydrolysis, which removes two ester groups to form tenofovir, while TAF undergoes hydrolysis by intracellular cathepsin A to form tenofovir.[12] Tenofovir is renally eliminated by glomerular filtration and tubular secretion. Clearance in the proximal tubule is controlled by membrane transport proteins. Tenofovir is actively transported into proximal renal tubular cells by organic anion transporters 1 and 2 (OAT1 and OAT2, encoded by *SLC22A6* and *SLC22A7*, respectively), and transported out of these cells by efflux transporters including multidrug resistant protein 2 and 4 (MRP2 and MRP4, encoded by *ABCC2* and *ABCC4*, respectively).[13,14] Genetic variants in these transporter genes have been postulated to increase risk of renal toxicity by favouring intracellular accumulation of tenofovir.

Studies of tenofovir toxicity in African populations have yielded inconsistent results.[15] Some found tenofovir-containing regimens to be without substantial renal toxicity,[16–18] while others found increased renal toxicity and recommend monitoring of renal function.[19,20] Candidate gene studies from various populations have sought to associate renal transporter polymorphisms with TDF renal toxicities among individuals treated for HIV, most of which did not include African populations. A study of 115 patients in Spain receiving TDF-containing regimens found an association between renal tubular dysfunction and homozygosity for *ABCC2* −24C/C (rs717620).[21] A study of 190 Japanese patients found an association between TDF-induced renal tubular dysfunction and two *ABCC2* polymorphisms (rs717620 and rs2273697).[22] Among 501 participants randomized to TDF-containing

regimens in AIDS Clinical Trials Group protocol A5202,[23] a *SLC22A2* polymorphism (rs3127573) was associated with more favourable creatinine clearance among African-American participants.[24] A study of Ghanaians receiving TDF-containing regimens suggested an associated between an *ABCC10* polymorphism (rs2125739) and worsening renal function.[25]

Because of inconsistent results from pharmacogenomic studies and the limited data regarding the pharmacogenetics of TDF toxicity among Africans, who have the highest genetic diversity worldwide, we evaluated whether laboratory markers of renal tubular injury were associated with polymorphisms in genes involved in TAF, TDF and tenofovir metabolism and transport among ART-naïve Southern Africans who had been randomized to initiate either TDF- or TAF-containing regimens in a prospective clinical trial.

Methods

Ethics

The present study was conducted in accordance with the Declaration of Helsinki and the ADVANCE protocol WRHI 060 (NCT03122262) received ethics and regulatory approvals from the Wits Human Research Ethics Committee (REF 160606B) and the South African Health Products Regulatory Authority (REF 20160620), respectively. Ethics approval for the pharmacogenetics sub-study was also granted by the University of Cape Town Health Sciences Human Research Ethics (REF 571/2019). Written informed consent for genetic research was obtained from study participants.

Study population

The ADVANCE study was a 96-week, randomized, phase 3 non-inferiority clinical trial which enrolled 1053 HIV-positive participants living in Johannesburg, South Africa. Participants in ADVANCE were randomly assigned 1:1:1 to receive: 1) TAF, dolutegravir, and emtricitabine; 2) TDF, dolutegravir, and emtricitabine; or 3) TDF, efavirenz, and emtricitabine.[26] Eligible participants were at least 12 years of age, weighed at least 40 kg, had creatinine clearances greater than 60 mL per minute, were ART naïve within 6 months before entry, were not pregnant, did not have active tuberculosis, and were not receiving anti-tuberculous therapy.[26] The ADVANCE trial has been

previously described in detail.[26] Among 702 participants randomized to receive either TAF, dolutegravir and emtricitabine or TDF, dolutegravir and emtricitabine, 340 (48%) consented for genetic research.

Study design

Genetic association analyses focused primarily on 23 polymorphisms that were identified in previous studies to be associated with TDF associated renal toxicity and 14 genes relevant to tenofovir disposition and tubular dysfunction (*ABCB1*, *ABCC10*, *ABCC2*, *ABCC4*, *ABCG2*, *AK2*, *AK3*, *CES1*, *CYP3A4*, *NME1*, *SLC22A2*, *SLC22A6*, *SLC22A8* and *SLC22A11*). Candidate gene studies have associated polymorphisms in *SCL22A6*, *ABCC2*, *ABCC4* with kidney tubular dysfunction[21,22,27] or reduced creatinine clearance.[28] We assessed estimated glomerular filtration rate, urinary retinol binding protein and urinary β_2-microglobulin as biomarkers of renal tubular toxicity. Secondary analyses explored all polymorphisms from the Pharmacogenomics Knowledgebase (PharmGKB) that were associated with any drug at levels of evidence of 1 or 2 (accessed 10 February 2021)[29], all NHGRI-EBI GWAS Catalog polymorphisms with p-values less than 5.0 x10^{-8} for any trait (accessed 10 February 2021)[30], and all polymorphisms in our imputed genome-wide genotype dataset.

Genetic Polymorphisms

Whole blood labelled with coded identifiers was stored and DNA extraction performed at the Sydney Brenner Institute for Molecular Bioscience at the University of the Witwatersrand, using the salting out method as described elsewhere.[31] Genotyping with the Illumina Infinium Multi-Ethnic Global BeadChip (MEGA^{EX}) was done at Vanderbilt Technologies for Advanced Genomics (VANTAGE) in Nashville, Tennessee, USA. Post-genotype quality control included sex checks, call rates by marker and sample, identity by descent (IDB) plots, assessment for batch effects, concordance between duplicate samples, and HapMap controls.

Quality control steps were performed using PLINK version 1.9.[32] Genotyping efficiency per participant was >95% in all samples. Markers with genotyping efficiency <95% were excluded, as were those with minor allele frequencies (MAF) <5%. We excluded 21 samples with overall

genotyping call rates <95%. After quality control, data were imputed using the TOPMed reference panel after transforming to genome build 38 using liftOver and stratification by chromosome to parallelize the imputation process.[33,34] For each chromosome in each phase, 100% concordance with genotyped data was assessed. Polymorphisms with imputation scores <0.3, genotyping call rates <99%, MAF <0.05, or Hardy-Weinberg Equilibrium (HWE) p-values $<1.0x10^{-8}$ were excluded. To control for population stratification, we used Eigenstrat/ Eigensoft package 6.0.1 to estimate principal components.

Clinical and laboratory data

Clinical data from the ADVANCE trial were collected at screening, enrolment, weeks 4 and 12, and every 12 weeks until week 96.[26] We selected for analysis three renal markers - estimated glomerular filtration rate (eGFR), retinol binding protein (RBP), and β_2-microglobulin (B2M). Estimated glomerular filtration rate was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[35] Change in eGFR from week 4 to week 48 ($\Delta eGFR_{4-48}$) was used in analysis. Week 4 was used as baseline because dolutegravir increases serum creatinine concentrations by inhibiting tubular secretion of creatinine, which plateaus by week 4.[36,37] The microprotein markers RBP and B2M were selected because their increases in urine are early indicators of renal tubular dysfunction or toxicity.[37] These markers were calculated as ratios to urine creatinine ($\Delta RBP:Cr_{0-48}$ and $\Delta B2M:Cr_{0-48}$, respectively).

The Shapiro–Wilk test was used to assess normality, with $P \leq 0.05$ considered statistically significant. Clinical data were not normally distributed and were described with frequencies, medians, and interquartile ranges (IQR). Natural logarithmic transformations were done on uRBP/Cr and uB2M/Cr to approximate normality.

Association analyses

Multivariable linear regression analyses were performed to evaluate associations between genetic polymorphisms and each renal outcome. Covariates included baseline age, sex, study arm (TAF or TDF), and screening body mass index (BMI), $\log_{10}$ plasma HIV-1 RNA, and CD4 T-cell count.

Bonferroni correction was used to account for multiple testing, with a cut-off of 0.05 divided by number of polymorphisms tested in each targeted analysis, and $P = 5.0x10^{-8}$ for genome-wide analyses.

Results

Of the 702 ART-naïve participants who were randomly assigned to the two dolutegravir-containing arms of ADVANCE, 336 were included in our study: 173 (51.5%) in the TAF group and 163 (48.5%) in the TDF group. All participants were Black Southern Africans (55.6% South African, 38.4%, Zimbabwean, and 6% from Lesotho, Malawi, Mozambique, Swaziland, or Zambia), median age was 32 years, most were female, and median BMI was 23.4 kg/m^2. Baseline characteristics were similar between the TAF and TDF groups (**Table 1**).

Of the 23 candidate polymorphisms, 14 passed quality control (QC) in our imputed genotype data. From PharmGKB, 173 polymorphisms were previously associated with at least one medication-related phenotype, and 31 passed QC in our imputed genotype data. From NHGRI-EBI GWAS Catalog, of 89,716 polymorphisms previously associated with any trait at $P < 5.0 x10^{-8}$ in at least one published study, 59,575 passed QC our imputed genotyped data. The remaining polymorphisms did not meet MAF, imputation score, or Hardy-Weinberg Equilibrium (HWE) cut-offs.

Genetic associations with change in estimated glomerular filtration rate

We first characterized associations with $\Delta eGFR_{4-48}$. Among 14 candidate polymorphisms previously reported to be associated with TAF, TDF and tenofovir disposition or renal toxicity, the lowest P-value for association with $\Delta eGFR_{4-48}$ was *ABCC4* rs899494 ($\beta = 3.49$, $P = 0.02$). Among 6304 polymorphisms in the candidate genes *ABCB1*, *ABCC10*, *ABCC2*, *ABCC4*, *ABCG2*, *AK2*, *AK3*, *CES1*, *CYP3A4*, *NME1*, *SLC22A2*, *SLC22A6*, *SLC22A8* and *SLC22A11* (± 50 kB), the lowest P-value for association with $\Delta eGFR_{4-48}$ was *ABCC4* rs4148481 ($\beta = 5.15$, $P = 1.5x10^{-4}$). Among 51 PharmGKB polymorphisms (of which 20 were also in the GWAS Catalog), the lowest P-value for association with $\Delta eGFR_{4-48}$ was *NAT2* rs1799930 ($\beta = -3.58$, $P = 0.026$). Among 59,575 polymorphisms previously associated with any GWAS Catalog trait, the lowest P-value for association with $\Delta eGFR_{4-}$

$_{48}$ was *NOL4L* rs1555133 (β = 8.49, P = 1.18 x10^{-5}). Considering genome-wide associations regardless of the GWAS Catalog, the lowest P-value for association with $\Delta eGFR_{4-48}$ was *COL27A1* rs1687402 (β = -9.4, P = 3.2 x 10^{-9}). In the above analyses for $\Delta eGFR_{4-48}$, only *COL27A1* rs1687402 withstood correction for multiple testing. The five lowest P-values for association with $\Delta eGFR_{4-48}$ from each prioritized analysis are presented in **Table 2**.

As a sensitivity analysis, we repeated the above analyses but based on change in eGFR from screening (rather than week 4) to week 48. In this analysis, no associations withstood correction for multiple testing. (Supplementary data available on request.)

Genetic associations with change in log-transformed urinary β_2-microglobulin:creatinine ratio

We next characterized associations with $\Delta B2M:Cr_{0-48}$. Among 14 candidate polymorphisms previously reported to be associated with TAF, TDF and tenofovir disposition or renal toxicity, the lowest P-value for association with $\Delta B2M:Cr_{0-48}$ was *ABCC4* rs1059751 (β = -0.37, P = 0.009). Among 6304 polymorphisms in the 14 candidate genes, the lowest P-value for association with $\Delta B2M:Cr_{0-48}$ was *PKD2* rs72659631 (β = -0.55, P = 8.7x10^{-4}). Among 51 PharmGKB polymorphisms, the lowest P-value for association with $\Delta B2M:Cr_{0-48}$ was intergenic polymorphism rs7297610 on chromosome 12 (β = -0.29, P = 0.01). Among 59,575 polymorphisms previously associated with any GWAS Catalog trait, the lowest P-value for association with $\Delta B2M:Cr_{0-48}$ was *ITG4* rs1143676 (β = -0.53, P = 1.4 x10^{-5}). Considering genome-wide associations regardless of the GWAS Catalog, the lowest P-value for association with $\Delta B2M:Cr_{0-48}$ was *ITG4* rs3770126 (β = -0.80, P = 4.5 x10^{-7}*)*. In the above analyses for $\Delta B2M:Cr_{0-48}$, no polymorphism withstood correction for multiple testing. The five lowest P-values for association with $\Delta B2M:Cr_{0-48}$ from each prioritized analysis are presented in **Table 3**.

Genetic associations with change in log-transformed urinary retinol binding protein: creatinine ratio

We next characterized associations with $\Delta RBP:Cr_{0-48}$. Among 14 candidate polymorphisms previously reported to be associated with TAF, TDF and tenofovir disposition or renal toxicity, the

lowest P-value for association with ΔRBP:Cr_{0-48} was *ABCC10* rs2125739 (β = 0.19, P = 0.07). Among 6304 polymorphisms in the 14 candidate genes, the lowest P-value for association with ΔRBP:Cr_{0-48} was intergenic polymorphism rs691857 on chromosome 9 (β = -0.35, P = 3.2×10^{-4}). Among 51 PharmGKB polymorphisms, the lowest P-value for association with ΔRBP:Cr_{0-48} was *CHRNA3* rs578776 (β = 0.22, P = 0.037). Among 59,575 polymorphisms previously associated with any GWAS Catalog trait, the lowest P-value for association with ΔRBP:Cr_{0-48} was intergenic polymorphism rs3848600 on chromosome 19 (β = -0.82, P = 1.3 $\times 10^{-5}$). Considering genome-wide associations regardless of the GWAS Catalog, the lowest P-value for association with ΔRBP:Cr_{0-48} was *CDH4* rs66494466 (β = -0.70, P = 3.4 $\times 10^{-8}$). In the above analyses for ΔB2M:Cr_{0-48}, only *CDH4* rs66494466 withstood correction for multiple testing. The five lowest P-values for association with ΔRBP:Cr_{0-48} from each prioritized analysis are presented in **Table 4**.

Associations by randomized study arm

Our study included randomized arm in ADVANCE (TAF or TDF) as a covariate. In our multivariable analyses, randomized study arm was not independently associated with any of the renal outcomes examined.

Discussion

We characterized genetic associations with renal tubular dysfunction among ART-naïve Southern African participants who were randomized to the two dolutegravir arms (plus either TAF and emtricitabine, or TDF and emtricitabine) of the ADVANCE clinical trial. Among 14 polymorphisms that we prioritized based on reported associations with TDF renal toxicity or plasma tenofovir exposure, we found nominal associations with two polymorphisms previously reported - *ABCC4* rs899494 with ΔeGFR$_{4-48}$, and *ABCC4* rs1059751 with ΔB2M:Cr_{0-48}. However, our associations were in the opposite direction than those previously reported, as discussed below. In addition, three polymorphisms in *COL27A1*, which encodes collagen type XXVII alpha 1 chain, were genome-wide associated with change in eGFR. An additional polymorphism in *CDH4* was genome-wide associated with ΔRBP:Cr_{0-48}.

Our primary analyses focused on polymorphisms previously reported to be associated with TDF-related renal toxicity in other populations. Prior studies have implicated multidrug resistance proteins, encoded by ABCC family members, in tenofovir clearance and TDF-related renal toxicity.[14,21,38] We found that *ABCC4* rs1059751 was nominally associated with ΔB2M:$Cr_{0\text{-}48}$ (P = 0.009). A previous report associated rs1059751 T→C (at position 4976 of *ABCC4*) with β_2-microglobulinuria in Thai patients treated with TDF-containing ART.[39] In that study, the rs1059751 C allele was associated with beta2-microglobulinuria, with allele frequency of 0.60 in cases and 0.48 in controls. In contrast, the present study associated the rs1059751 G allele (equivalent to C allele in the Thai study) with a decrease in B2M (β = -0.37), with a MAF of 0.19. The negative association indicates that participants with the minor G allele in our study were less likely to experience renal tubular dysfunction (increased β_2-microglobulinuria). We also found that *ABCC4* rs899494 G→A was nominally associated $\Delta$$eGFR_{4\text{-}48}$ (P = 0.02). This polymorphism was previously associated with renal phosphorus wasting in Spanish patients treated with TDF-containing ART.[21] In that study, the CC genotype was associated with worsening of the renal marker. However, for the present study, the A allele (equivalent to T allele in the Thai study) was associated with greater eGFR decline, which may be indicative of renal tubular dysfunction. We did not replicate associations with *ABCC2* rs2273697 and *ABCC10* rs2125739, which were previously reported to be associated with altered amino acid excretion [21] and with kidney tubular dysfunction, respectively.[38]

In the present study, several *COL27A1* polymorphisms were genome-wide associated with change in $\Delta$$eGFR_{4\text{-}48}$. This gene encodes collagen type XXVII alpha 1 chain,[40] and mutations in *COL27A1* have been associated with Steel syndrome, knee osteoarthritis and Achilles tendinopathy.[41–43] Although *COL27A1* is expressed in renal tubules,[44] it is unclear how this gene would contribute mechanistically to tenofovir renal toxicity.

An additional polymorphism in *CDH4* gene was associated with change in ΔRBP:$Cr_{0\text{-}48}$. *CDH4* encodes cadherin 4, a calcium-dependent cell-cell adhesion glycoprotein which plays a vital role in the development of various organs, including the retina, brain, gastrointestinal tract, pancreas, and kidney.[45] Dysregulation of *CDH4* has been associated with several human cancers, including renal

cell carcinoma.[46] CDH4 is overexpressed in kidney clear cell carcinoma, but it is unclear how *CDH4* gene would contribute mechanistically to tenofovir renal toxicity.

To identify novel polymorphisms associated with the renal outcomes, we leveraged imputed genome-wide data against the considerable knowledge generated by prior genetic association studies represented in PharmGKB and the GWAS Catalog. We reasoned that polymorphisms associated with at least one drug-related phenotype in PharmGKB with levels of evidence of 1 or 2 (as described in Methods) or with any trait in the GWAS Catalog at $P < 5.0 \times 10^{-8}$ in at least one published study, would most likely be true positives in the present study. We did not identify any such associations that withstood correction for multiple testing.

Our study has some limitations. A larger sample size would have increased power to detect associations with modest effect size, or associations with infrequent variants. We did not adjust for concomitant drugs that are potentially nephrotoxic, which may have obscured genetic associations. The study was performed exclusively in Black Southern Africans living with HIV, who were previously shown to be at lower risk of renal tubular dysfunction[47] and TDF-associated renal tubulopathy;[48] our results can therefore not be extrapolated to other populations. Additionally, we did not adjust for population stratification in our cohort. This could have obscured genetic associations because of allelic differences between different subpopulations in Southern Africans included in the study. The relatively short exposure (48 weeks) and use of an unboosted third agent in this young population may have contributed to the minimal effects of tenofovir on renal tubular function, and thus our ability to identify genetic associations with the three measures of renal tubular dysfunction.

In conclusion, our study found associations with two polymorphisms previously reported to be associated with TDF-induced renal toxicity, although in our study the associations were in the opposite directions than previously reported. We also identified genome-wide significant associations with renal markers and polymorphisms in *COL27A1* and *CDH4*. These findings contribute to our understanding of tenofovir-related renal toxicity.

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Table 1: Baseline characteristics of consented study participants in TAF, dolutegravir, and emtricitabine or TDF, dolutegravir, and emtricitabine arms

Characteristics median [IQR [b]]	**TAF/DTG/FTC (n = 173)**	**TDF/DTG/FTC (n = 163)**	**P-value[a]**
Age (years)	32 [27 to 38]	32 [27 to 37]	0.90
Sex, n (%)			0.88
Female	109 (63%)	104 (64%)	
Male	64 (37%)	59 (36%)	
Screening BMI (Kg/m^2)	23.6 [20.6 to 26.5]	23.1 [20.1 to 27.1]	0.98
Screening CD4 count (cells/µl)	334 [170 to 502]	276 [159 to 424]	0.22
Screening HIV viral load (log copies/mL)	4.5 [3.8 to 4.9]	4.4 [3.8 to 4.9]	0.79
Renal markers at baseline; med [IQR]			
eGFR week 0 (mL/min/1.73m^2)	135.8 [125.4 to 142.2]	135.8 [124.4 to 142.7]	0.74
eGFR week 4 (mL/min/1.73m^2)	123 [104.7 to 134.2]	117 [100.5 to 131.9]	0.05
uRBP week 0 (µg/L)	59 [29 to 128]	74 [30 to 140]	0.71
uRBP:Cr week 0 (µg/mmol)	5.6 [3.3 to 11.4]	6.0 [3.2 to 11.5]	0.77
uB2M week 0 (µg/L)	165.5 [87.5 to 295]	129 [70 to 302]	0.80
uB2M:Cr week 0 (µg/mmol)	0.014 [0.008 to 0.025]	0.014 [0.007 to 0.025]	0.66

[a]P-value for comparison between study groups was calculated using Wilcoxon rank-sum. P-value for sex was determined using Z-tests for proportions. [b]abbreviations: IQR, interquartile range; TAF, tenofovir alafenamide fumarate; TDF, tenofovir disoproxil fumarate; DTG, dolutegravir; FTC, emtricitabine; eGFR, estimated glomerular filtration rate; RBP, retinol binding protein; B2M, Beta 2-Microglobulin; Cr, creatinine.

Table 2: Polymorphisms with lowest P-values for association with change in eGFR from week 4 to week 48

Polymorphism	Gene	Chromosome	Reference allele	Variant allele	MAF	Beta	P-value
Candidate SNPs[a] (n = 14 polymorphisms)							
rs899494	*ABCC4*	13	G	A	0.26	3.49	0.021
rs1045642	*ABCB1*	7	G	A	0.12	-3.68	0.067
rs1557070	*ABCC4*	13	G	A	0.31	-2.55	0.081
rs1751034	*ABCC4*	13	T	C	0.36	1.81	0.19
rs2125739	*ABCC10*	6	T	C	0.27	1.84	0.19
Candidate Genes[b] (n = 6304 polymorphisms)							
rs4148481	*ABCC4*	13	G	A	0.47	5.15	1.46E-4
rs55828993	*intergenic*	10	T	G	0.24	6.04	1.89E-4
rs17013743	*PKD2*	4	A	G	0.11	8.37	1.91E-4
rs11817399	*ABCC2*	10	T	G	0.11	8.04	2.31E-4
rs115787791	*intergenic*	10	T	A	0.11	8.04	2.41E-4
PharmGKB and GWAS Catalog[c] (n = 51 polymorphisms)							
rs1799930	*NAT2*	8	G	A	0.24	-3.58	0.026
rs776746	*CYP3A5*	7	T	C	0.19	-3.96	0.028
rs8050894	*VKORC1*	16	C	G	0.24	2.98	0.053

rs1800566	*NQO1*	16	G	A	0.18	3.36	0.062
rs1051266	*SLC19A1*	21	T	C	0.28	-2.78	0.065
GWAS Catalog[d] (n = 59,575 polymorphisms)							
rs1143676	*ITGA4*	2	A	G	0.27	-0.53	1.39E-5
rs6508210	*DCC*	18	C	A	0.34	0.48	2.30E-5
rs3770104	*ITGA4*	2	T	C	0.27	-0.50	2.83E-5
rs7245004	*DCC*	18	A	C	0.22	0.54	3.57E-5
rs10031777	*ZAR1*	4	C	T	0.37	0.44	6.36E-5
Genome-wide genotype data[e]							
rs1687402	*COL27A1*	9	A	G	0.23	-9.36	3.17E-9
rs1687400	*COL27A1*	9	C	T	0.23	-9.12	1.23E-8
rs1626295	*COL27A1*	9	C	T	0.23	-9.13	1.25E-8
rs309421	*intergenic*	5	A	G	0.40	7.14	7.97E-8
rs4958396	*LOC105378239*	5	G	T	0.29	7.24	3.03E-7

[a]Significance threshold was 0.0036 for a subset of 14 SNPs. [b]Significance threshold was $7.9x10^{-6}$ for a subset of 6304 SNPs. [c]Significance threshold was $9.8x10^{-4}$ for a subset of 51 SNPs. [d]Significance threshold was $8.4x10^{-7}$ for a subset of 59 575 SNPs. [e]Significance threshold was $5x10^{-8}$ for the genome-wide analysis.

Table 3: Polymorphisms with lowest P-values associated with change in log-transformed β_2-microglobulin adjusted for creatinine from screening to week 48

Polymorphism	Gene	Chromosome	Reference allele	Variant allele	MAF	Beta	P-value
Candidate SNPs[a] (n = 14 polymorphisms)							
rs1059751	*ABCC4*	13	A	G	0.19	-0.37	0.0087
rs2273697	*ABCC2*	10	G	A	0.14	-0.30	0.05
rs2125739	*ABCC10*	6	T	C	0.27	0.16	0.17
rs1557070	*ABCC4*	13	G	A	0.31	0.15	0.22
rs1751034	*ABCC4*	13	T	C	0.36	0.14	0.23
Candidate Genes[b] (n = 6304 polymorphisms)							
rs72659631	*PKD2*	4	C	T	0.12	-0.55	0.00087
rs1766896	*intergenic*	13	T	C	0.45	-0.37	0.0013
rs2992914	*intergenic*	13	C	T	0.37	0.38	0.0014
rs9516561	*intergenic*	13	C	T	0.37	0.38	0.0016
rs186032556	*intergenic*	11	C	A	0.07	-0.67	0.0016
PharmGKB and GWAS Catalog[c] (n = 51 polymorphisms)							
rs7297610	*intergenic*	12	C	T	0.36	-0.29	0.012
rs578776	*CHRNA3*	15	A	G	0.40	0.25	0.031
rs4673993	*ATIC*	2	T	C	0.07	0.42	0.074
rs12979860	*IFNL4*	19	T	C	0.41	0.20	0.077
rs489693	*intergenic*	18	C	A	0.44	-0.20	0.077

GWAS Catalog[d](n = 59,575 polymorphisms)							
rs3848600	*Intergenic*	19	C	T	0.07	-0.82	1.25E-5
rs7247962	*Intergenic*	19	T	C	0.07	-0.87	1.45E-5
rs12714199	*LOC105374845*	2	C	T	0.49	0.41	3.91E-5
rs2952858	*LINC02465*	4	A	G	0.34	0.41	4.84E-5
rs2006092	*GGT1*	22	G	A	0.17	-0.52	7.38E-5
Genome-wide genotype data[e *]							
rs3770126	*ITGA4*	2	A	G	0.14	-0.80	4.47E-7

[a]Significance threshold was 0.0036 for a subset of 14 SNPs. [b]Significance threshold was 7.9x10^{-6} for a subset of 6304 SNPs. [c]Significance threshold was 9.8x10^{-4} for a subset of 51 SNPs. [d]Significance threshold was 8.4x10^{-7} for a subset of 59 575 SNPs. [e]Significance threshold was 5x10^{-8} for the genome-wide analysis. [*]Only 1 SNP associated with ΔB2M:Cr$_{0-48}$ had $P < 1\text{x}10^{-6}$ during GWAS SNP filtering.

Table 4: Polymorphisms with lowest P-values associated with change in log retinol binding protein adjusted for creatinine from screening to week 48

Polymorphism	Gene	Chromosome	Reference allele	Variant allele	MAF	Beta	P-value
Candidate SNPs[a] (n = 14 polymorphisms)							
rs2125739	*ABCC10*	6	T	C	0.27	0.19	0.068
rs3742106	*ABCC4*	13	A	C	0.42	-0.15	0.17
rs1557070	*ABCC4*	13	G	A	0.31	0.13	0.24
rs1128503	*ABCB1*	7	G	A	0.08	0.21	0.27
rs11568626	*SLC22A6*	11	C	T	0.06	-0.18	0.40
Candidate Genes[b] (n = 6304 polymorphisms)							
rs691857	*intergenic*	9	A	G	0.44	-0.35	3.20E-4
rs410437	*intergenic*	9	A	G	0.32	0.37	4.55E-4
rs10737365	*intergenic*	1	A	C	0.07	-0.63	5.93E-4
rs4149181	*SLC22A8*	11	G	A	0.45	0.31	2.46E-3
rs113523901	*intergenic*	17	C	T	0.12	0.48	2.79E-3
PharmGKB and GWAS Catalog[c] (n = 51 polymorphisms)							
rs578776	*CHRNA3*	15	A	G	0.40	0.22	0.037
rs7412	*APOE*	19	C	T	0.17	-0.24	0.079
rs1800629	*intergenic*	6	G	A	0.15	-0.25	0.085
rs3766951	*OPRD1*	1	T	C	0.31	0.18	0.092
rs6295	*intergenic*	5	C	G	0.42	-0.17	0.099

GWAS Catalog[d] (n = 59,575 polymorphisms)							
rs3848600	*Intergenic*	19	C	T	0.07	-0.82	1.25E-5
rs7247962	*Intergenic*	19	T	C	0.07	-0.87	1.45E-5
rs12714199	*LOC105374845*	2	C	T	0.49	0.41	3.91E-5
rs2952858	*LINC02465*	4	A	G	0.34	0.41	4.84E-5
rs2006092	*GGT1*	22	G	A	0.17	-0.52	7.38E-5
Genome-wide genotype data[e]							
rs66494466	*CDH4*	20	G	A	0.18	-0.70	3.43E-8
rs7960855	*intergenic*	12	T	C	0.33	0.53	3.78E-7
rs10004174	*intergenic*	4	T	C	0.15	0.71	5.60E-7
rs9472842	*PHACTR1*	6	C	T	0.10	-0.82	5.72E-7
rs9472865	*PHACTR1*	6	T	C	0.10	-0.79	8.18E-7

[a]Significance threshold was 0.0036 for a subset of 14 SNPs. [b]Significance threshold was $7.9x10^{-6}$ for a subset of 6304 SNPs. [c]Significance threshold was $9.8x10^{-4}$ for a subset of 51 SNPs. [d]Significance threshold was $8.4x10^{-7}$ for a subset of 59 575 SNPs. [e]Significance threshold was $5x10^{-8}$ for the genome-wide analysis.

Appendices

JAC author guidelines

Article types and format

All documents should be double spaced, and the margins should not be excessively wide. A clear, legible single font (which is readily available internationally) and point size should be employed throughout. For symbols, please use the 'insert symbol' function and ONLY select characters from the 'normal text' subset. All submitted articles should be line numbered (using continuous line numbers). To do this in Word, use File, Page Setup, Layout, Line Numbers and select continuous line numbering. Please DO NOT insert page numbers (as the pdf proof created by the online submission system will automatically be page numbered).

All articles should include a title page comprising: article title; author names and their affiliations (each affiliation address must be given separately and in full); telephone, fax and e-mail contact details for the corresponding author; and a short running title. In addition, all articles must include a Funding section (if reporting original research) and a Transparency declarations section.

Article titles. All articles reporting the results of original research must have a descriptive title. For example ‘Effect of streptomycin in tuberculosis’ is acceptable; ‘Streptomycin cures tuberculosis’ is not acceptable. Viewpoints, which are expressions of opinion, are permitted to have declarative titles. Please note that claims of priority are not permitted in article titles as such claims are impossible to verify; only history will reveal the first example. For instance ‘First NDM-1 Escherichia coli isolated in Andorra’ would not be permitted. Authors are permitted to indicate in the article that, to the best of their knowledge, a finding is the first of its kind.

Original articles and Brief reports must have a structured synopsis. The headings for the structured synopsis are as follows: Background (optional), Objectives, Patients and methods (or Methods), Results, and Conclusions.

Original articles. There is a limit of 3500 words in the main text of the article (everything from the Introduction to the end of the Discussion). Papers must be written as concisely as possible. Original articles are divided into the following sections: Synopsis (250 words maximum), Introduction, Materials (or Patients) and methods, Results, Discussion, Acknowledgements, Funding, Transparency declarations and References. Repetition of content between sections must be avoided. A combined Results and Discussion section is acceptable.

Authorship

The authorship of the paper should be confined to those who have made a significant contribution to the design and execution of the work described. In the case of clinical trials/randomized control trials it is compulsory for the contribution of each author to be clearly stated in the Transparency declarations section, after the information on conflicts of interest. Authors of other types of article may indicate the contribution made by each author if they wish.

Ethics

All articles in JAC describing research in humans or animals must include an 'Ethics' heading as the first section in the Patients and methods or Methods section. Authors must include in this section all relevant statements regarding approvals, licences, informed consent and so on, as applicable.

Research involving humans

Authors must indicate in the Ethics section whether the research was conducted in accordance with the Declaration of Helsinki and national and institutional standards. If approval was obtained from an Ethics Committee the authors must clearly name the ethics committee responsible if more than one institution is involved. The approval/reference number must be listed in the Ethics section of the article. Written informed consent must be obtained from study participants and the existence of this consent must be stated in the article. Authors must supply the relevant approval numbers from Ethics committees or other bodies.

Patient privacy. Patients have a right to privacy. Any information that might result in identification of individuals must be omitted, especially if it is not directly clinically relevant. Patient age, sex, admission dates and co-morbidities should be removed as far as possible. If it is possible that a patient could be identified, the authors must obtain written informed consent from the individual(s) concerned and state that this has been obtained in the article. Publication consent forms should be retained by the authors and not supplied to the Journal. If the patient is deceased, the next of kin should be contacted. If consent cannot be obtained the authors must explain the circumstances briefly in the article, as well as in detail in the covering letter. In rare circumstances where relevant clinical details mean that the patient can be identified, the patient/next of kin must be shown the manuscript before submission and made aware as part of the informed consent process that the article may appear on the internet.

Funding

ALL papers submitted to JAC reporting original research MUST include a 'Funding' section. This section should appear after the 'Acknowledgements' section. Details of all funding sources for the work in question must be given.

Authors must list any internal funding. If no specific funding has been received then this should be clearly stated; equally if data have been generated as part of the routine work of an organization, this too should be stated. Ongoing financial support for any of the authors should also be included under the Funding heading. If a professional medical writer or similar service was involved in the origin or preparation of a manuscript and this support was funded, the source must be declared in the Funding section.

Sources of funding may of course still be thanked in the Acknowledgements section, but should not be listed again in the Transparency declarations (see below), unless there is an important reason for doing so. For example if the funder played any decision-making role in the research this must be stated.

The following rules should be followed:

The sentence should begin: 'This work was supported by …'

The full official funding agency name should be given, i.e. ‘the National Cancer Institute at the National Institutes of Health’ or simply 'National Institutes of Health' not ‘NCI' (one of the 27 sub institutions) or 'NCI at NIH’ (full RIN-approved list of UK funding agencies)

Grant numbers should be complete and accurate and provided in brackets as follows: ‘(grant number ABX CDXXXXXX)’

Multiple grant numbers should be separated by a comma as follows: ‘(grant numbers ABX CDXXXXXX, EFX GHXXXXXX)’

Agencies should be separated by a semi-colon (plus ‘and’ before the last funding agency)

Where individuals need to be specified for certain sources of funding the following text should be added after the relevant agency or grant number 'to (author initials)'.

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Conflicts of interest have the potential to affect authors, referees and Editors (including Senior Editors and the Editor-in-Chief). JAC has the following systems in place to deal with conflicts of interest:

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Transparency declarations

In the interests of openness, ALL papers submitted to JAC MUST include a ‘Transparency declarations’ section (which should appear at the end of the paper, before the ‘References’ section). We suggest authors concentrate on transparency declarations (i.e. conflicts of interest) of a financial nature, although relevant non-financial disclosures can also be made. Authors should consider making a declaration if they answer 'Yes' to any of the following questions:

Have you in the period of research leading up to this publication accepted any of the following from an organization (including government departments or granting bodies) that may in any way be financially affected by the conclusions of your article (e.g. reimbursement for attending a symposium, a fee for

speaking, a consultancy fee, funds for research other than directly for this work, funds for a member of staff, any other substantial material benefit)?

Do you directly own any stocks or shares in a company that might be financially affected by the conclusions of your article?

Has the funder of the research played any decision-making role in the design, execution, analysis or reporting of the research?

Have you received the assistance of a professional medical writer or similar service? [The precise role of the writer or service in the origin or preparation of the manuscript must be declared and we recommend that the name of the writer (and their agency where applicable) or the service is provided.]

Have you accepted any reimbursement for preparing your article?

Authors should either include appropriate declarations or state ‘None to declare’. Importantly, the declarations should be kept as concise as possible, should avoid giving financial details (e.g. sums received, numbers of shares owned etc.), and should be restricted to declarations that are specific to the paper in question. Authors will of course need to consider whether or not the transparency declarations need to be amended when revisions are submitted.

The burden of responsibility rests with all authors, who must ensure that appropriate declarations are included. The corresponding author will be responsible for obtaining the relevant information from all of their co-authors. By signing a submission form each author is stating that they have made any necessary transparency declaration. All authors should carefully consider the embarrassment and potential damage to their reputation that could result should they fail to declare an interest that is revealed subsequently.

If only some authors need to make a declaration it must be made clear that the remaining authors have nothing to declare, for example:

'A.B. has received funds for speaking at symposia organized on behalf of Panacea Ltd and has also received funds for research from Panacea. C.D. is a member of the Panacea advisory board for fantastazole. All other authors: none to declare.'

All papers submitted to JAC must include a transparency declarations section; papers that do not include such a section will not enter the review process; they will be returned to the corresponding author so that the appropriate section can be added. Following resubmission the paper will then be progressed to peer review.

In the case of clinical trials/randomized control trials it is compulsory for the contribution of each author to be clearly stated in the Transparency declarations section, after the information on conflicts of interest. Authors of other types of article may indicate the contribution made by each author if they wish.

Spelling

British spelling should be used. Spelling should follow that of the Oxford Dictionary for Scientific Writers and Editors and where this gives no guidance the Concise Oxford Dictionary. Spelling of drug names should conform with that given in the latest edition of the British National Formulary (published by the British Medical Association and the Royal Pharmaceutical Society of Great Britain and available online), but please note that JAC will continue to use methicillin (not meticillin).

Abbreviations

Non-standard abbreviations should be defined at the first occurrence and introduced only where multiple use is made. See this document for abbreviations that may be used without definition, as well as antimicrobial abbreviations (which may be used in Tables and Figures).

Data Citation

Availability of Data and Materials

Where ethically feasible, JAC strongly encourages authors to make all data and software code on which the conclusions of the paper rely available to readers. We suggest that data be presented in the main

manuscript or additional supporting files, or deposited in a public repository whenever possible. Information on general repositories for all data types, and a list of recommended repositories by subject area, are available on the Research Data Policy page.

Data Citation JAC supports the Force 11 Data Citation Principles and requires that all publicly available datasets be fully referenced in the reference list with an accession number or unique identifier such as a digital object identifier (DOI). Data citations should include the minimum information recommended by Data Cite:

[dataset]* Authors, Year, Title, Publisher (repository or archive name), Identifier

*The inclusion of the [dataset] tag at the beginning of the citation helps us to correctly identify and tag the citation. This tag will be removed from the citation published in the reference list.

UNIVERSITY OF CAPE TOWN
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26 August 2019

HREC REF: 571/2019

Dr P Sinxadi
Division of Clinical Pharmacology
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Dear Dr Sinxadi

PROJECT TITLE: TFV_PK_PG_01/2019- PHARMACOGENETICS OF TENOFOVIR ADMINISTERED AS ALAFENAMIDE OR DISPROXIL FUMARATE PRO-DRUGS IN HIV-POSITIVE BLACK SOUTH AFRICANS (MSC IN CLINICAL PHARMACOLOGY _ MISS S MATEZA)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study including the following documentation: -

1. PI generated synopsis
2. Protocol Version 1.0 dated 08 August 2019
3. Optional Pharmacogenomics ICF Version 1.0 dated 30 May 2018
4. Main Study Protocol WRHI 060 Version 3.0 dated 12 December 2017
5. ICF for Storage and Future research Version 3.0
6. SAHPRA Approval
7. Budget Summary
8. Material TRF Agreement
9. Ethics Approval letter -WRHI 060 protocol version 300 dated 15 Dec 2017
10. Ethics approval letter - Pharmacogenomics ICF version 1.0 dated 30 May 2018

Approval is granted for one year until the 30 August 2020.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the student: Miss Somila Mateza will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
NHREC-registration number: REC-210208-007

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

Name: Somila Mateza MTZSOM001 Version 1.0
Supervisor: Dr Phumla Sinxadi
Co-supervisors: Prof David Haas and Prof Gary Maartens

Pharmacogenetics of Tenofovir Administered as Alafenamide or Disoproxil Fumarate Pro-drugs in HIV-positive Black South Africans

A. Specific Aims and Hypothesis

The ADVANCE study in South Africa is an open label randomised, non-inferiority, phase 3 study in which 1110 HIV-positive participants were randomly assigned to one of three treatment groups: group 1 received dolutegravir (DTG), tenofovir alafenamide fumarate (TAF), and emtricitabine (FTC); group 2 received DTG, tenofovir disoproxil fumarate (TDF) and FTC; group 3 received efavirenz (EFV), TDF, and FTC over 96 weeks. The present proposal is an analysis of the pharmacogenetic data of participants in the DTG arms of the main study who are on TAF or TDF and have given separate consent for genetic sampling.

Aim 1: To investigate the associations between novel or selected single nucleotide polymorphisms (SNPs) in the candidate genes involved in metabolism and transport of TAF or TDF, and plasma concentrations of tenofovir.

We hypothesize that novel or known SNPs in the candidate genes *ABCB1, ABCC10, ABCC2, ABCC4, ABCG2, AK2, AK3, CatA, CES-1, CYP3A4, NME1, SLC22A6, SLC22A8 and SLC22A11*, will be associated with interindividual differences in the pharmacokinetics of tenofovir (TFV) in an HIV-positive South African population.

Aim 2: To investigate the associations between selected SNPs and change in creatinine clearance (CrCl) from baseline.

We hypothesize that SNPs in the candidate genes *ABCB1, ABCC10, ABCC2, ABCC4, ABCG2, AK2, AK3, CatA, CES-1, CYP3A4, NME1, SLC22A2, SLC22A6, SLC22A8 and SLC22A11*, including known loss-of-function or gain-of-function SNPs, will be associated with change in CrCl from baseline to 24 and 48 weeks in an HIV-positive South African population.

B. Background and Significance

The estimated number of people living with HIV in South Africa is 7.2 million and 61% of these individuals are receiving antiretroviral therapy (ART).[1] The standardised national ART regimens for adults and adolescents in S.A is tenofovir disoproxil fumarate (TDF) + emtricitabine (FTC) or lamivudine (3TC) + efavirenz (EFV)[2], which follows the 2015 World Health Organisation (WHO) recommendation of a combination of two nucleoside reverse transcriptase inhibitors (N(t)RTIs) and a non-nucleoside reverse transcriptase inhibitor (NNRTI) or an integrase strand transfer inhibitor (INSTI).[3] The 2019 WHO updated guidelines strongly recommend dolutegravir (DTG) in combination with a N(t)RTI as the backbone of the first-line regimen.[4]

TDF is a once-daily nucleotide analogue reverse transcriptase inhibitor with activity against HIV-1 and HIV-2.[5] TDF is a disoproxil diester prodrug that is rapidly converted to TFV in plasma.[5] It undergoes esterase hydrolysis, which removes the two ester groups. Cleavage of the first ester forms a monoester intermediate, while cleavage of the second ester yields TFV.[5] In contrast, tenofovir alafenamide fumarate (TAF), a newer prodrug of tenofovir, has a different activation mechanism than TDF.[6, 7] TAF carboxyester bond is cleaved via hydrolysis by protease cathepsin A (*CatA*), and carboxylesterase 1 (*CES-1*).[6, 8, 9] This initial step releases TAF-alanine that is further hydrolysed to TFV.[6] Tenofovir, derived from either TDF or TAF, is then phosphorylated intracellularly by the cellular nucleotide kinase, adenylate kinase, to the monophosphate intermediate, and then rapidly converted by nucleoside diphosphate kinase to the active diphosphate form.[5, 6] Tenofovir diphosphate (TFV-DP) is a competitive inhibitor

of HIV-1 reverse transcriptase that competes with deoxyadenosine 5′-triphosphate and terminates the growing DNA chain by incorporating into DNA during HIV transcription.[5] TAF has higher oral bioavailability compared to TDF. The use of TAF prodrug produces 90% lower plasma concentrations of TFV and four times higher concentrations of intracellular TFV-DP, when compared to TDF.[10, 11] TDF is given in a standard 300 mg/day dose, whereas TAF is recommended to be given in a lower dose of 25 mg in an unboosted regimen or 10 mg in a boosted regimen,[12, 13] which will decrease the costs of manufacturing. A further advantage of TAF over TDF is that TAF has a lower rate of renal and bone toxicity.[5, 8]

Upon oral administration, TDF enters proximal tubule cells through the human organic anion transporters 1 & 3 (hOAT 1&3), which are encoded for by *SLC22A6* and *SLC22A8* respectively, and exits the tubular lumen across the apical membrane by the multidrug-resistant proteins 2 & 4 (MRP 2 & 4) which are coded for by *ABCC2* and *ABCC4* respectively.[14, 15] TDF is also a substrate of breast cancer resistance protein (BCRP) which is an efflux transporter found in the kidneys.[16] Previous studies have shown genetic polymorphisms of these transporters to be associated with higher tenofovir exposure.[17, 18] Candidate gene studies have identified polymorphisms in *SCL22A6, ABCC2, ABCC4* to be associated with higher TFV concentrations,[17, 19, 20] and kidney tubular dysfunction[20-22] or reduced creatinine clearance (CrCl).[23] *SCL22A6* rs11568626 has been shown to be associated with higher TFV concentrations and was only found in an African sub-population.[19] In a genome-wide association study among patients randomised to receive TDF/FTC in the ACTG A5202 study, no genome-wide significant associations with change in CrCl were found.[24] However, in a candidate SNP analyses stratified by population, rs3127573 in *SLC22A2* was significantly associated with a positive 6 month change in CrCl among African Americans.[24]

Africans have the largest genetic diversity, and some of the important polymorphisms in genes encoding for metabolising enzymes and drug transporters are found in higher frequencies in African populations.[19, 24] Polymorphisms in renal transporter genes may alter tenofovir disposition and may be associated with renal and bone toxicity. Polymorphisms in genes encoding for TAF metabolizing enzymes, such as *CatA* and *CES-1*, may be associated with interindividual differences in TFV concentrations. Therefore, it is important to investigate polymorphisms in an African population that may affect the accumulation and toxicity of tenofovir given as TDF or TAF. We propose to assess genetic determinants of TFV exposure and renal toxicity in participants randomised to TAF or TDF.

C. Preliminary Studies/Progress Report

This is a sub-study of the ADVANCE study in South Africa. The ADVANCE study is an open label randomised, non-inferiority (10% non-inferiority margin), phase 3 study of TAF + DTG + FTC, compared with TDF + DTG + FTC, and with TDF + EFV + FTC, over 96 weeks in HIV-positive patients starting first-line ART. The primary endpoint of ADVANCE is 48-week viral load, which will be reported at the 2019 International AIDS Society conference. The present study is an analysis of the pharmacogenetic data of participants in the main study who are on the TAF + DTG + FTC arm, or the TDF + DTG + FTC arm, and have given separate consent for genetic sampling.

D. Research Design and Methods

D1. Study Design

This study is an analysis of the pharmacogenetic data from ADVANCE participants on TAF or TDF and have given separate written informed consent for genetic sampling. We will conduct a genetic association study using data and specimens from these patients, where we will investigate the associations of SNPs in *ABCB1, ABCC10, ABCC2, ABCC4, ABCG2, AK2, AK3, CatA, CES-1, CYP3A4, NME1, SLC22A2, SLC22A6, SLC22A8 and SLC22A11* and plasma concentrations of tenofovir.

D2. Study Population

Approximately 1110 male and female patients with HIV-1 who were eligible for first-line ART were randomly assigned in a 1:1:1 ratio (approximately 370 patients per treatment group) to treatment group 1 (TAF + DTG + FTC); or treatment group 2 (TDF + DTG + FTC); or treatment group 3 (TDF + EFV + FTC). Of the 1110 study participants, 90 to 120 were planned to be in the 12 to 19-year age group.

Inclusion criteria: Age ≥ 12 years and weighed ≥ 40 kg. Documented laboratory diagnosis of infection with HIV-1 with plasma HIV-1 RNA (VL) ≥ 500 copies/mL. Calculated creatinine clearance (CrCl) > 60 mL/min in > 18 years old OR > 80 mL/min in ≤ 18 years old. Ability to understand the full nature and purpose of the study and to comply with the requirements of the entire study. The present analysis will include individuals who participated in the ADVANCE study and consented to the genetic sub-study.

Exclusion criteria: For ADVANCE, patients who received any antiretrovirals within 6 months before start of the study or receiving treatment with any other investigational drug or device. Pregnant patients at time of screening visits and those with active tuberculosis and/or are on antituberculous therapy at time of the screening were also excluded. Participants that had a strong likelihood of relocating far enough to make access to the study site difficult, with a history or presence of allergy to the study drugs and unstable liver disease were also excluded.

D3. Confidentiality

This study will only use deidentified data. The investigators will not have any access to any participant-identifying information, and no effort will be made to identify participants. The ADVANCE protocol was approved at the University of the Witwatersrand (WRHI 060). Ethical approval for the present proposal will be obtained from the University of Cape Town Human Research Ethics Committee.

D4. Genotyping

Genomic sampling was performed at week 36 in all participants who consented. Whole blood (10 mL) was stored and DNA extraction performed at Sydney Brenner Institute for Molecular Bioscience at the University of the Witwatersrand, using the salting out method as described by Miller *et. al*.[25] Samples will be labelled with coded identifiers. Stored DNA will be genotyped with the Illumina Infinium Multi-Ethnic Global BeadChip (MEGAEX), at Vanderbilt Technologies for Advanced Genomics (VANTAGE) in Nashville, Tennessee, USA. Targeted SNPs not genotyped by MEGAEX may be genotyping using complementary techniques. Quality control will be performed using the software program PLINK.[26] Samples that have a call rate <1%, identity by descent (IBD) >0.25, unresolved sex discrepancies and have a z score <4 standard deviations and exceptionally low or high heterozygosity will be excluded. Analysis of genomic factors associated with antiretroviral drug exposures will initially focus on

known functional SNPs of the relevant genes involved in the absorption, distribution, metabolism and elimination of TAF and TDF.

D5. Pharmacogenomics and pharmacokinetics analysis

Plasma for pharmacokinetic analysis in ADVANCE are collected at weeks 24 and 48. The samples will be centrifuged, and plasma stored at -80°C for analysis. Plasma tenofovir concentrations will be quantified using liquid chromatography with tandem mass spectrometry (LC/MC/MS). Analysis of polymorphisms associated with tenofovir drug exposure will initially focus on known functional polymorphisms, to investigate if SNPs in renal anion transporter genes and metabolizing enzymes, such as *ABCB1, ABCC10, ABCC2, ABCC4, ABCG2, AK2, AK3, CatA, CES-1, CYP3A4, NME1, SLC22A2, SLC22A6, SLC22A8 and SLC22A11* will predict tenofovir exposure. The full dataset, including additional imputed polymorphisms based on an African reference panel, will be used for subsequent genome-wide association analysis for factors associated with plasma exposure and/or toxicity from tenofovir.

D6. Limitations

The ADVANCE study was designed to enrol approximately 740 patients in treatment group 1 (TAF + DTG + FTC) and treatment group 2 (TDF + DTG + FTC), with 370 patients in each group. Only half the anticipated number of patients provided written informed consent for further genetic analysis, therefore decreasing the sample size for this study.

D7. Timetable

Activity	2019			2020			
	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Obtain ethics committee approvals							
Genotype DNA samples							
Obtain pharmacokinetics data							
Perform association analyses							
Write up and submission							

E. Sample Size Justification and Statistical Analysis Plan

The proposed analyses will include 350 participants, based on the data availability. A forward stepwise linear regression approach will be used to test for association between each genetic variant and TFV estimated C_{min}. C_{min} values will be log transformed if they do not adhere to normality. To assess the relationship of polymorphisms inherited together, Haploview will be used to construct linkage disequilibrium blocks. Chi-square tests will be conducted to test for deviation from Hardy-Weinberg equilibrium. P-values <0.05 will be considered significant. Associations will be identified by stepwise linear regression, which will include principal component vectors to adjust for genetic ancestry. The Bonferroni method will be used to adjust for multiple testing.

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