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High rate of K65R for ART naïve patients with subtype C HIV infection failing a TDF-containing first-line regimen in South Africa

Henry Sunpath,

McCord Hospital, Durban, South Africa

Baohua Wu,

Rollins School of Public Health, Emory University, Atlanta, Georgia

Michelle Gordon,

Nelson Mandela School of Medicine, Durban, South Africa

Jane Hampton,

McCord Hospital, Durban, South Africa

Brent Johnson,

Rollins School of Public Health, Emory University, Atlanta, Georgia

Yunus Moosa,

Nelson Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa

Claudia Ordonez,

McCord Hospital, Durban, South Africa

Daniel R. Kuritzkes¹,

Division of Infectious Diseases, Brigham and Women's Hospital, Boston, MA

Vincent C. Marconi, and

Emory University School of Medicine, Atlanta, Georgia Rollins School of Public Health, Emory University, Atlanta, Georgia

SARCS Team Group Authors

Abstract

Objective—We sought to determine the rate of the K65R mutation in patients receiving tenofovir (TDF)-based antiretroviral treatment (ART) with subtype C HIV infection.

Design—Retrospective cohort study.

[Corresponding author: henrysunpath@yebo.co.za and current address: 45 McCord Road, Overport, South Africa] .

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Henry Sunpath [Study design, manuscript preparation, and research supervision]

Baohua Wu [Data analysis and manuscript review]

Michelle Gordon [Drug resistance testing and study design]

Jane Hampton [Study design and critical review of manuscript]

Brent Johnson [Data analysis and manuscript review]

Yunus Moosa [Study design and Critical review of manuscript]

Claudia Ordonez [Study design and implementation]

Daniel R. Kuritzkes [Study design and critical review of manuscript]

Vincent C. Marconi [Study design, Manuscript preparation, and supervision of study]

SARCS Team Group Authors [Contributed to initial Study Design and Data Collection] [Helga Holst, Sifiso Shange, Roma Maharaj,

Melisha Pertab, Sabelo Dladla (McCord Hospital, Sinikithemba Clinic, Durban, South Africa)]

Methods—All patients initiated on d4T+3TC or TDF+3TC plus a NNRTI at McCord Hospital in Durban, South Africa had their charts reviewed. All patients with virologic failure (VF), defined as a viral load (VL) > 1000 copies/mL after 5 months of a first ART regimen, had genotypic resistance testing performed prospectively using a validated in-house assay. Important resistance mutations were selected based upon published mutations in subtype B virus in the Stanford HIV Drug Resistance (DR) Database.

Results—A total of 585 patients were initiated on TDF-containing first-line ART from August 3, 2010 to March 17, 2011. Thirty-five (6.0%) of these patients had VF and 23/33 (69.7%) of the VF patients had the K65R mutation. The median (IQR) for the baseline CD4 count was 105 cells/uL (49-209) and VL at VF was 47,571 copies/mL (20,708-202,000). During the same time period, 53 patients were initiated on d4T-containing regimens. Two (3.8%) of these patients had VF and 1 of the VF patients had the K65R mutation.

Conclusions—Preliminary data show very high rates (>65%) of K65R for patients failing TDF-based first-line regimens at McCord hospital with few additional NRTI mutations compared to subtype B. These rates may reflect faster *in vivo* selection, longer time on a failing regimen, or transmitted DR.

Keywords

first-line antiretroviral therapy; virologic failure; HIV-1 drug resistance; K65R; tenofovir; South Africa; subtype C virus

INTRODUCTION

Tenofovir (TDF) has been part of first-line antiretroviral therapy (ART) for most developed countries since 2001. Because of potency, durability, tolerability, favorable pharmacokinetics and drug interaction profile, TDF quickly emerged as one of the two most commonly prescribed NRTI's for antiretroviral (ARV) naïve subjects.[1] In areas endemic for hepatitis B virus (HBV), TDF has the added benefit of possessing activity against HBV. In April, 2010 TDF was introduced as part of first-line ART in the South African national treatment plan, replacing the more toxic ARV, stavudine (d4T).[2] To date, there are no published reports on the effectiveness of TDF as part of first-line ART in this setting.

The reverse transcriptase (RT) mutation K65R results in a four-fold decrease in TDF susceptibility and is selected by TDF, didanosine (ddI), d4T and abacavir (ABC).[3] The K65R mutation has been reported in 7-15% of patients failing d4T-, ddI- or zidovudine-(AZT) containing first or second-line ART in South Africa, where subtype C accounts for most HIV-1 infections, compared to 2-5% of patients in parts of the world where infection with subtype B dominates.[4-11] *In vitro* studies provide some evidence for more rapid selection of K65R in subtype C virus.[12-14] We previously reported the virologic effectiveness and prevalence of drug resistance (DR) mutations after initiating first-line ART in South Africa.[15] In this study, we sought to determine the virologic outcomes and rate of K65R emergence amongst patients with virologic failure after initiating TDF-containing ART as first-line treatment in a clinic in Durban, South Africa.

METHODS

A retrospective analysis of HIV-1 DR was conducted at McCord Hospital, which has been treating patients with ART since 2002. All patients initiated on d4T+3TC or TDF+3TC plus a NNRTI from August 3, 2010 to March 17, 2011 were included in the analysis. Virologic failure (VF) was defined as a VL > 1000 copies/mL after 5 months of a first ART regimen.

Genotypic resistance testing was performed prospectively as part of a larger research study on all patients with VF using a validated in-house assay. This study was approved by the respective ethics committees at McCord Hospital and by the institutional review boards at Emory University in Atlanta, Georgia and Partners HealthCare Systems in Boston, Massachusetts. All VF patients provided written informed consent for study participation. Important resistance mutations were selected based upon the published mutations on subtype B virus in the Stanford HIV DR Database. The prevalence of drug-resistant virus in the samples tested was reported with 95% CIs, calculated based upon normal approximation of binomial distribution. RT and protease resistance mutations were also reported.

Data collected at baseline included age, gender, prior AIDS-defining illnesses, ART treatment history, CD4 cell count and plasma HIV-1 RNA level at time of regimen failure. Analyses were performed using SAS software, version 9.3 (SAS Institute). All tests of statistical significance were 2-sided; associations with $P < 0.05$ were considered to be statistically significant. Continuous variables were compared using the Wilcoxon rank-sum test; categorical variables were compared using the χ^2 test or Fisher's exact test. Univariate and multivariate logistic regression were used to identify variables associated with the presence of K65R. Variables known to be associated with study outcomes, as well as independent variables exhibiting an association with study outcomes in the bivariate analysis at $P \leq 0.1$ or odds ratios of ≥ 1.5 (or ≤ 0.6), were advanced into the multivariate analyses.

RESULTS

Of 585 patients initiated on TDF-containing first-line ART, 35 (6.0%) experienced VF. Baseline characteristics of these patients are presented in Table 1. The median age (range) was 37.3 yrs (31.2-45.0), 45.7% of the patients were women, 88.6% were on efavirenz, and the median duration of TDF and ART was 5.3 (5.0-6.1) and 5.7 (5.2-15.1) months, respectively. The median number of prior AIDS-defining illnesses was 1 (1.0-2.0), with tuberculosis being the most common (74.3%). The median (IQR) CD4 count at study entry was 105 cells/uL (49-209); median VL at study entry (VF) was 47,571 copies/mL (20,708-202,000). Two patients had virus that could not be amplified for resistance genotyping. All isolates were subtype C. Twenty-three (69.7%) of the 33 patients with VF and amplifiable virus had the K65R mutation. Additional mutations frequencies are described in table 2.

In contrast, 53 patients initiated a d4T-containing regimen during the same time period. Two (3.8%) of these patients had VF and one had the K65R mutation. Additional RT mutations with the K65R included K103N and V106M. Median duration of ART appeared different between the two groups but no statistical test is reported due to low sample size in the group initiating a d4T-containing regimen. For those commencing d4T the median duration of ART was 13.7 (5.3-22.2) months compared to 5.7 (5.2-15.1) months for TDF. Ten (28.6%) of the 35 TDF patients had prior d4T and had been previously switched to TDF for toxicity (not virologic failure) after a median of 24.8 months (15.1-30.1) on d4T. One of these patients could not be genotyped. Of the remaining 9 patients, 4 (44.4%) had the K65R mutation. Therefore if these 9 patients were excluded from the 33 total patients with amplifiable virus on TDF, then 19 (79.2%) had the K65R mutation.

The following factors were evaluated, using regression analysis, to determine their association with K65R amongst patients failing a TDF-containing first-line ART: age, gender, regimen, CD4 count, viral load, duration of ART and prior AIDS-defining illnesses. No associations were found in univariate regression and therefore multivariate models were not constructed.

DISCUSSION

ART has been shown to be effective in the treatment of HIV-1 infection, regardless of the viral subtype. However, specific DR mutations can emerge at different rates and the prevalence of some DR mutations differs depending upon the subtype.[7][16] An understanding of DR patterns among non-B subtype infections may help to optimize the selection of first-line ART in order to limit the emergence of DR. Resistance pathways, which could compromise the use of second-line ARVs through cross-resistance, also vary among different subtypes.[17] This concern may be increased in developing countries where formularies are limited. [5] However, mutations found in combination with K65R in our study either have a minor effect on susceptibility to AZT or enhance susceptibility to AZT (eg.L74V, Y181C, M184V as well as K65R). Therefore we expect that AZT would remain quite active against such viruses.

During the initial ART rollout at McCord Hospital, the K65R mutation in patients failing first-line therapy for at least six months was reported in only three patients out of a total of 147 (2.6%).[6] Although some of these patients had prior suboptimal ART, most were naive and failing on a d4T, ddI- or AZT-based regimen. In that study, 97.4% of patients had subtype C virus. Of concern, HIV-1 from approximately 20% of patients in areas in which subtype C is endemic carries the K65R mutation, the K70E mutation, or both after experiencing VF of a d4T- or ddI-based ART regimen.[5] K65R was also detected in 7 to 15% of patients in South Africa who did not have a response to first- or second-line regimens with d4T, ddI, or AZT as the nucleoside backbone.[2] Some of the differences in these rates of acquisition of K65R or thymidine analog mutations (TAMs) are doubtless due to treatment regimens and disease stage, as well as limited access to viral-load testing in many developing countries.

In this analysis, the findings of relatively fewer M184V mutations and absence of TAMs provides some evidence of the antagonism that exists between these mutations and K65R. Although the M184V mutation may have emerged early, variants with this mutation would have been overcome by the more fit K65R variants which likely emerged later. Recent data suggest that increased rates of K65R acquisition in subtype C may be due to the nature of the subtype C RNA template. In particular, RT of subtype C viruses may be especially prone to pausing events at codon 65 due to a poly-adenine region that allows for misalignment, misincorporation, strand transfer, insertions, deletions and recombinations, thereby facilitating the acquisition of K65R during reverse transcription.[4] Ultrasensitive pyrosequencing methods have also shown that K65R can be selectively transmitted as minority species to some populations that have not yet received antiretroviral therapy.[8] Transmission of these variants could jeopardize not only first-line ART but also pre- and post-exposure prophylaxis strategies containing TDF.[9,10]

There are a few limitations of this study that should be noted. First, there may be a selection bias associated with the study population as is common in retrospective or cross-sectional studies. Patients who were lost to follow up, died or changed service providers prior to study entry would not have been included in this analysis. Additionally, the relatively small sample size does not allow for effective comparisons to be made with the concurrent d4T group and could be an explanation for why risk factors for K65R emergence could not be determined. However, it is unlikely that a larger sample size would significantly alter the prevalence of the K65R mutation for patients receiving TDF and comparisons can be made with the historical reports of virologic failure and K65R for patients receiving d4T-containing ART in this same setting.

K65R is uncommon among patients with subtype B who have received either tenofovir or a combination of tenofovir and emtricitabine as part of triple antiretroviral-drug therapy.[2] The data reported here show very high rates (>65%) of K65R for patients failing TDF-based first-line regimens at McCord with few additional NRTI mutations. These rates may reflect faster *in vitro* selection, longer time on a failing regimen, or transmitted DR. Larger numbers of patients and longer follow-up are required to determine whether the emergence of K65R in subtype C is consistent and clinically relevant in this setting. Although risk factors were not identified in this analysis, larger studies may reveal which patients could be at risk for developing K65R. It is an urgent global priority to optimize treatment strategies for HIV infection, regardless of geographic locale. Moreover, this study provides additional evidence that the provision of VL monitoring and genotypic resistance testing, both before and after ART, needs to be expanded to include all developing countries.[18, 19]

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Table 1
Baseline characteristics of patients with virologic failure during first-line ART with and without tenofovir

Characteristic		All patients (n=37)	TDF-containing (n=35)	No TDF use (n=2)
Age, Mean $\pm$ SEM [IQR]		37.3 [31.2 – 45.0]	37.3 [31.2 – 45.0]	37.1 [28.1 – 46.1]
Women (%)		18/37 (48.6%)	16/35 (45.7%)	2/2 (100.0%)
EFV (%)		33/37 (89.2%)	31/35 (88.6%)	2/2 (100.0%)
Median duration of ART (months) [IQR] by TDF/D4T		5.3 [5.0 – 6.1]	5.3 [5.0 – 6.1]	7.1 [4.7 – 9.5]
Median duration of ART (months) [IQR]		5.7 [5.2 – 15.1]	5.7 [5.2 – 15.1]	13.7 [5.3 – 22.2]
Median CD4 count at virologic failure (cells/ul) [IQR]		107.0 [49.0 – 209.0]	105.0 [49.0 – 209.0]	173.0 [173.0 – 173.0];
CD4 cell count category (%)	0-49 cells/ul	8/37 (21.6%)	8/35 (22.9%)	0/2 (0.0%)
	50-99 cells/ul	4/37 (10.8%)	4/35 (11.4%)	0/2 (0.0%)
	100-199 cells/ul	8/37 (21.6%)	7/35 (20.0%)	1/2 (50.0%)
	200-349 cells/ul	5/37 (13.5%)	5/35 (14.3%)	0/2 (0.0%)
	>350 cells/ul	2/37 (5.4%)	2/35 (5.7%)	0/2 (0.0%)
Median plasma viral load at virologic failure (copies/ml) [IQR]		45,817 [19212 – 194,000]	47,571 [20,708 – 202,000]	23,173 [2365 – 43,981]
Viral load category (copies/ml) (%)	400-4,999	5/37 (13.5%)	4/35 (11.4%)	1/2 (50.0%)
	5,000-29,999	7/37 (18.9%)	7/35 (20.0%)	0/2 (0.0%)
	30,000-99,999	13/37 (35.1%)	12/35 (34.3%)	1/2 (50.0%)
	> 100,000	11/37 (29.7%)	11/35 (31.4%)	0/2 (0.0%)
Prior AIDS-defining illness		1.0 [1.0 – 2.0]	1.0 [1.0 – 2.0]	1.0 [1.0 – 1.0]
TB		28/37 (75.7%)	26/35 (74.3%)	2/2 (100.0%)
KS		1/37 (2.7%)	1/35 (2.9%)	0/2 (0.0%)
Toxoplasmosis		2/37 (5.4%)	2/35 (5.7%)	0/2 (0.0%)
Cryptococcal Meningitis		1/37 (2.7%)	1/35 (2.9%)	0/2 (0.0%)

TDF – tenofovir, EFV – efavirenz, ART – antiretroviral therapy, TB – tuberculosis, HSV – herpes simplex virus, KS – Kaposi's Sarcoma, IQR – interquartile range; For a sample of size 2, the median and IQR are the mean and range, respectively; 1 of 2 patients in the d4T group is missing CD4 cell count.

Table 2
Frequency of selected resistance mutations in the reverse transcriptase and protease genes for individuals treated with tenofovir in first line antiretroviral therapy

Mutation	No. (%) of patients (n = 33)	95% Confidence Interval^c
NRTI resistance		
M41L	1 (3.0)	0.08-15.76
A62V	0 (0)	0.00-10.58
K65R	23 (69.7)	51.29-84.41
D67N	3 (9.1)	1.92-24.33
K70R/E	3 (9.1)	1.92-24.33
L74V	2 (6.1)	0.74-20.23
V75I	2 (6.1)	0.74-20.23
F77L	0 (0)	0.00-10.58
Y115F	7 (21.2)	8.98-38.91
F116Y	0 (0)	0.00-10.58
Q151M	0 (0)	0.00-10.58
M184V/I	9 (27.3)	13.30-45.52
L210W	0 (0)	0.00-10.58
T215Y	0 (0)	0.00-10.58
T215F	0 (0)	0.00-10.58
K219Q/E/N/R	1 (3.0)	0.08-15.76
TAM 1	1 (3.0)	0.08-15.76
TAM 2	4 (12.1)	3.40-28.20
TAM 1 and 2 ^a	0 (0)	0.00-10.58
Total with any TAMs ^b	5 (15.2)	5.11-31.90
Any NRTI	33 (100.0)	89.42-100.00
NNRTI resistance		
L100I	2 (6.1)	0.74-20.23
K103N	8 (24.2)	11.09-42.26
V106A	1 (3.0)	0.08-15.76
V106M	18 (54.5)	36.35-71.89
Y181C/I	7 (21.2)	8.98-38.91
Y188C/L/H	8 (24.2)	11.09-42.26
G190A/S	5 (15.2)	5.11-31.90
P225H	1 (3.0)	0.08-15.76
M230L	0 (0.0)	0.00-10.58
Any NNRTI	32 (97.0)	84.24-99.92
PI resistance		

Mutation	No. (%) of patients (n = 33)	95% Confidence Interval^c
D30N	0 (0.0)	0.00-10.58
V32I	0 (0.0)	0.00-10.58
L33F/I	1 (3.0)	0.08-15.76
M46I/L	0 (0.0)	0.00-10.58
I47V/A	0 (0.0)	0.00-10.58
G48V	0 (0.0)	0.00-10.58
I50V	0 (0.0)	0.00-10.58
I54V	0 (0.0)	0.00-10.58
V82A/T/F/S	0 (0.0)	0.00-10.58
I84V	0 (0.0)	0.00-10.58
L90M	1 (3.0)	0.08-15.76
Any PI	2 (6.1)	0.74-20.23

Patient Identification Number	Reverse Transcriptase Selected	Protease Selected Mutations
CAS35	K65R, L74V, G109E, Y115F	None
CAS39	K65R, K70R/E, V75I, V106M, Y115F, Y188C	None
CAS73	K65R, Y115F, V106M, M184V	None
CAS96	K65R, L100I, M184V, Y318F	None
CAS107	K65R, D67N, V75I, V106A, Y115F, K219E	None

NOTE. NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor; TAM, thymidine analog mutation; RT, reverse transcriptase; PR, protease.

^aPercentage of patients with at least 1 mutation in each of these 2 pathways: TAM 1 (41L, 210W, and 215Y) and TAM 2 (67N, 70R, 215F, and 219Q/E/N/R).

^bTotal percentage of patients with TAM 1 and/or TAM 2.