ABSTRACT

Objective: We sought to understand if the rate of K65R was increased for patients with subtype C receiving TDF-based ART compared to reports on patients with subtype B.

Design: Retrospective cohort study.

Methods:  All patients who initiated on AZT+3TC, D4T+3TC or TDF+3TC plus a NNRTI at McCord Hospital in Durban, South Africa had their chart reviewed. Virologic failure (VF) was defined as a VL > 1000 copies/mL after 6 months of a first ART regimen. Genotypic resistance testing was performed prospectively on all patients with VF using a validated in-house assay. Significant resistance mutations were selected based upon the published mutations in the Stanford HIV Database.

Results: A total of 585 patients were initiated on TDF-containing first-line ART from August 3, 2010 to March 17, 2011. 33 (5.6%)35(6.0%) of these patients had VF and 18 (54.5%)23(65.7%) of VF patients had the K65R mutation. The median/IQR for the baseline CD4 count was 94 cells/uL (49-160)105[49-209] and VL at VF was 47,000 copies/mL (30,212-267,537)47571[20708-202000]. In contrast, 232 patients were initiated on D4T-containing regimens during an earlier time period. 4 (1.7%)(5/232=2.2%) of these patients had VF and 12 patient had the K65R.

Conclusions: Early data show very high rates (>50%) of K65R for patients failing TDF-based first-line regimens at McCord with few additional NRTI mutations. These rates may be related to the faster *in vitro* selection, longer time on a failing regimen, or transmitted DR.

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Running Headline: K65R after TDF-based first-line ART in Subtype C

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SARCS Team Group Authors [Contributed to initial study design]

[Bruce D. Walker (Massachusetts General Hospital, Boston, MA), Helga Holst, Sifiso Shange, Melisha Pertab (McCord Hospital, Sinikithemba Clinic, Durban, South Africa)]

Potential conflicts of interest

1. D.R.K. is a consultant to, or has received research funding from Abbott, Boehringer-

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**Key words:** first-line antiretroviral therapy, virologic failure, HIV-1 drug resistance, K65R, tenofovir, resource-limited settings, South Africa

INTRODUCTION:

Tenofovir (TDF) has been used as part of first-line antiretroviral therapy (ART) for most developed countries since 2001. Because of potency, durability and tolerability, TDF quickly became one of the two most commonly prescribed NRTI for antiretroviral (ARV) naïve individuals beginning ART.[[1](#_ENREF_1)] In April 2010, tenofovir (TDF) was introduced as part of first-line ART in the South Africa national roll-out plan replacing the more toxic ARV stavudine (D4T). To date, there are no reports on the effectiveness of using TDF for 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) and abacavir (ABC). K65R has been reported in 7-15% of patients failing D4T, DDI or zidovudine (AZT) containing first or second-line ART in South Africa compared to 2-5% for patients with Subtype B (including individuals treated with TDF). Data from *in vitro* studies have provided some evidence for a more rapid selection of K65R in subtype C virus.[[2-4](#_ENREF_2)] We previously reported the virologic effectiveness and prevalence of drug resistance (DR) after first-line ART in South Africa.[[5](#_ENREF_5)] In this study, we sought to determine the virologic outcomes and rate of K65R emergence for patients 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 on ART since 2002. All patients initiated on AZT+3TC, D4T+3TC or TDF+3TC plus a NNRTI during the period of 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 6 months of a first ART regimen. Genotypic resistance testing was performed prospectively as part of a larger research study for all patients with VF using a validated in-house assay. This study was approved by the respective ethics committees at McCord and by the institutional review board at Emory University in Atlanta, Georgia. Significant resistance mutations were selected based upon the published mutations in the Stanford HIV Database. The prevalence of drug-resistant virus in the samples tested was reported with 95% CIs, calculated based on normal approximation of binomial distribution. The number of reverse-transcriptase inhibitor and protease inhibitor resistance mutations was 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 x9.3 (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 χ2 test or Fisher’s exact test. Univariate and multivariate logistic regression were used to identify variables associated with the presence of K65R. Variables that had a known association with outcomes, as well as those independent variables that exhibited an association with outcomes in bivariate analysis at P < 0.1 or odds ratios of > 1.5 (or < 0.6), were advanced into multivariate analyses.

RESULTS:

A total of 585 patients were initiated on TDF-containing first-line ART. Thirty-three (5.6%) 35(6.0%)of these patients had VF and 18 (54.5%) (23/35=65.7%)of 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). Additional mutations found in patients having the K65R mutation included Y115F (57 patients), L74V (12), Y115FS (10), M184V (39), T69D/N (23), K70T (1), V179D (45), Y181C (67), V106M (1318), Y188C (35), G190A/E (69), V108I (12), A98G (12), K103N (68). In contrast, 232 patients were initiated on D4T-containing regimens during an earlier time period. Four (1.7%)Five(2.2%) of these patients had VF and onetwo of these patients had the K65R mutation.

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.[[6](#_ENREF_6)] 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.

Indeed, we have limited knowledge of resistance mutations in non-B subtypes of HIV-1 and their clinical relevance, despite the fact that more than 90% of patients with HIV-1 infection worldwide have non– subtype B variants of HIV. Furthermore, resistance pathways in different subtypes may compromise the use of specific second-line regimens through cross-resistance. This concern may be increased in developing countries where formularies are limited.[[4](#_ENREF_4)]

In this same population 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%).[[5](#_ENREF_5" \o "Marconi, 2008 #266)] Although some of these patients had prior suboptimal ART, most were naïve and failing on a D4T, DDI or AZT-based regimen. Of concern, patients in areas in which subtype C was endemic had a high rate (approximately 20%) of the K65R multinucleoside resistance mutation, the K70E mutation, or both mutations after receiving drug regimens based on D4T or DDI.[[4](#_ENREF_4)] 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](#_ENREF_2)]

Some of these differences in rates of acquisition of K65R or TAMs are doubtless due to treatment regimens and disease stage, as well as access to viral-load testing in many developing countries. This is underscored by the fact that timely introduction of second-line therapy after failure of first-line therapy, which is commonly associated with the M184V or NNRTI mutations, should prevent the emergence of thymidine analog mutations (TAMs) or K65R. In this analysis, the notable absence of M184V and TAMs provides some evidence of the antagonism that exists between these mutations and K65R. Although the M184V mutation may have emerged early, they 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 and not, for example, to the subtype origin of the viral reverse transcriptase. In particular, subtype C viruses may be especially prone to pausing events at codon 65 produced by a poly-Adenine stretch that allows for misalignment, misincorporation, strand transfer, insertions, deletions and recombinations, facilitating the acquisition of K65R during reverse transcription.[[3](#_ENREF_3)]Indeed, ultrasensitive pyrosequencing methods have also shown that K65R can be selectively transmitted as minority species in some populations that have not received antiretroviral therapy.[[7](#_ENREF_7)]

It may be speculated that varying emergence rates for DR mutations could have important implications for the durability of treatment efficacy and this may be true only for the use of substandard drug regimens. The K65R mutation emerges faster in subtype C than in subtype B. K65R is uncommon among patients with subtypes B and C who have received either tenofovir or a combination of tenofovir and emtricitabine as part of triple antiretroviral-drug therapy.[[2](#_ENREF_2)] Although this reflects the use of well-tolerated, effective drugs that have long intracellular half-lives and that act in combination to suppress viral replication and prevent the emergence of resistance mutations, larger numbers of patients and follow-up will be required to determine whether any consistent effect of the emergence of K65R in subtype C is clinically relevant.[[7](#_ENREF_7)]Of an additional concern for the rapid selection of K65R in subtype C HIV-1 is related to transmission these variants which could compromise not only first-line ART but also pre- and post-exposure prophylaxis strategies containing TDF.[[8](#_ENREF_8), [9](#_ENREF_9)] It is an urgent global priority to optimize treatment strategies for HIV infection, regardless of geographic locale. Policy-makers have to optimize the selection of first-line regimens and limit the acquisition of resistance.

Early data show very high rates (>50%) of K65R for patients failing TDF-based first-line regimens at McCord with few additional NRTI mutations. These rates may be related to the faster *in vitro* selection, longer time on a failing regimen, or transmitted DR. Genotypic resistance testing both before and after ART needs to be expanded to include all developing countries. Larger numbers of patients and follow-up will be required to determine whether the emergence of K65R in subtype C is consistent and clinically relevant in this setting.

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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=40)* | *TDF-containing (n=35)* | *No TDF use (n=5)* | *p value* |
| --- | --- | --- | --- | --- | --- |
| Age, Mean ± SEM [IQR] |  | 37.2[30.7 - 44.8] | 37.3[31.2 - 45.0] | 31.3[28.2 - 40.6] | 0.466 |
| Women(%) |  | 20/40( 50.0%) | 16/35( 45.7%) | 4/5( 80.0%) | 0.342 |
| EFV(%) |  | 36/40( 90.0%) | 31/35( 88.6%) | 5/5(100.0%) | 1.000 |
| Median duration of ART (months) [IQR] by TDF/D4T |  | 5.3[5.0 - 6.5] | 5.3[5.0 - 6.1] | 29.5[9.5 - 36.0] | 0.030 |
| Median duration of ART (months) [IQR] |  | 6.0[5.2 – 21.8] | 5.7[5.2 – 15.1] | 29.5[22.2 - 36.0] | 0.048 |
| Median CD4 count at virologic failure (cells/ul) [IQR] |  | 108.0[49.0 - 193.0] | 105.0[49.0 - 209.0] | 142.0[111.0 - 173.0] | 0.567 |
| CD4 cell count category (%) | 0-49 cells/ul | 8/40( 20.0%) | 8/35( 22.9%) | 0/5( 0.0%) | 0.337 |
|  | 50-99 cells/ul | 4/40( 10.0%) | 4/35( 11.4%) | 0/5( 0.0%) |  |
|  | 100-199 cells/ul | 9/40( 22.5%) | 7/35( 20.0%) | 2/5( 40.0%) |  |
|  | 200-349 cells/ul | 5/40( 12.5%) | 5/35( 14.3%) | 0/5( 0.0%) |  |
|  | >350 cells/ul | 2/40( 5.0%) | 2/35( 5.7%) | 0/5( 0.0%) |  |
| Median plasma viral load at virologic failure (copies/ml) [IQR] |  | 47057[17716 - 186000] | 47571[20708 - 202000] | 43981[2365 - 88000] | 0.442 |
| Viral load category (copies/ml) (%) | 400-4,999 | 6/40( 15.0%) | 4/35( 11.4%) | 2/5( 40.0%) | 0.317 |
|  | 5,000-29,999 | 7/40( 17.5%) | 7/35( 20.0%) | 0/5( 0.0%) |  |
|  | 30,000-99,999 | 14/40( 35.0%) | 12/35( 34.3%) | 2/5( 40.0%) |  |
|  | > 100,000 | 12/40( 30.0%) | 11/35( 31.4%) | 1/5( 20.0%) |  |
| Prior AIDS-defining illness |  | 1.0[1.0 - 2.0] | 1.0[1.0 - 2.0] | 1.0[1.0 - 2.5] | 0.801 |
|  | Cryptococcus | 1/40( 2.5%) | 1/35( 2.9%) | 0/5( 0.0%) | 1.000 |
|  | TB | 31/40( 77.5%) | 26/35( 74.3%) | 5/5(100.0%) |  |
|  | HSV | 1/40( 2.5%) | 0/35( 0.0%) | 1/5( 20.0%) | 0.292 |
|  | KS | 2/40( 5.0%) | 2/35( 5.7%) | 0/5( 0.0%) |  |
|  | TB | 3/40( 7.5%) | 2/35( 5.7%) | 1/5( 20.0%) |  |
|  | Toxoplasmosis | 1/40( 2.5%) | 1/35( 2.9%) | 0/5( 0.0%) |  |

Wilcoxon and Chi-square, Fisher’s tests used for two group comparisons (>1 resistance mutation vs. no resistance); TDF – tenofovir, EFV – efavirenz, ART – antiretroviral therapy, TB – tuberculosis, HSV – herpes simplex virus, KS – Kaposi’s Sarcoma, IQR – interquartile range

**Table 2. Factors associated with K65R amongst patients failing a tenofovir-based first-line ART.**

| *Factor* | *.* | *Odds Ratio* | *95% CI* |
| --- | --- | --- | --- |
| Age |  | 0.93 | [0.84-1.03] |
| Gender | Male vs Female | 3.00 | [0.50-23.14] |
| Regimen(EFV) | EFV Yes vs No | 0.67 | [0.01-9.88] |
| CD4 Count | 50-99 cells/ul vs 0-49 cells/ul | 1.18 | [0.04-94.10] |
|  | 100-199 cells/ul vs 0-49 cells/ul | 1.00 | [0.05-19.26] |
|  | 200-349 cells/ul vs 0-49 cells/ul | 1.18 | [0.04-94.10] |
|  | >350 cells/ul vs 0-49 cells/ul | 0.60 | [0.00-11.40] |
| Viral Load | >100,000 vs 400-4,999 | 0.60 | [0.00-5.84] |
|  |  | 0.87 | [0.00-8.09] |
|  |  | 2.27 | [0.00-23.15] |
| Duration of ART |  | 1.01 | [0.98-1.04] |
| Prior AIDS-defining illness |  | 0.96 | [0.28-3.30] |

ART – antiretroviral therapy