Viral Failure and High K65R in Kenyan Patients on Tenofovir Based First-Line Therapy

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I. Background and Objectives

Tenofovir (TDF)-based first-line antiretroviral therapy (ART) is globally recommended by the World Health Organization (WHO) for HIV-infected patients requiring therapy. Effects of switching from prior non-TDF to TDF-based regimens on ART success are unknown.

The TDF-signature K65R mutation has been reported at different rates across HIV-1 subtypes; 0-17% in subtype B in the US and Europe; 12-70% in subtype C in South Africa; and 57-59% in subtype G and circulating recombinant form (CRF)02_AG in Nigeria.

High rates of K65R in HIV-1 subtype C are associated with a poly-A template in reverse transcriptase (RT) positions 64-66. Other factors postulated to promote development of K65R in resource-limited settings include being on a prior non-TDF based first-line ART regimen, advanced disease, decreased frequency of viral load (VL) monitoring and delayed transition to second-line therapy.

In Kenya, HIV-1 infection is diverse with predominant subtypes A (50-80%), D (10-20%) and C (5-15%). Studies on acquired drug resistance on first-line ART failure in Kenya prior to TDF introduction report low levels (0-13%) of K65R.

We evaluated treatment failure and drug resistance in Kenyan patients on TDF-based first-line ART, and compared outcomes between patients with and without prior non-TDF based first-line ART exposure.

II. Methods

We enrolled patients receiving first-line ART at the main Academic Model Providing Access to Healthcare (AMPATH) clinic, located at the Moi Teaching and Referral Hospital (MTRH) in Eldoret, Kenya (Figure 1). AMPATH provides treatment to 140,000 HIV positive patients, with MTRH, its largest clinic, managing 27,820 adults (≥18 years), 68% (18,963) of whom have started ART.

Enrollment criteria included (i) HIV infection, (ii) Age ≥18 years, (iii) on WHO-recommended first-line ART for >6 months, including TDF + lamivudine (3TC)/emtricitabine (FTC) + efavirenz (EFV)/nevirapine (NVP), with or without prior first-line ART including zidovudine (AZT)/stavudine (D4T) + 3TC/FTC + EFV/NVP, (iv) ART self reported adherence >50% in the past month and the past 7 days, and (v) informed consent.

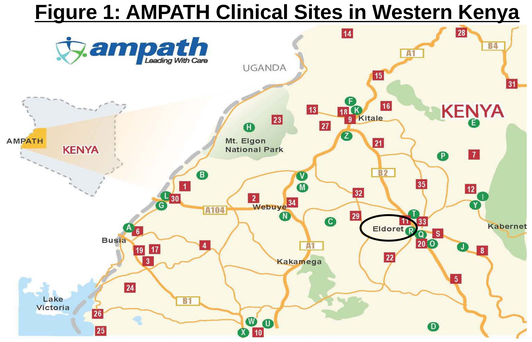
Demographic, clinical and laboratory patient data were collected from charts and interviews.

Patients had VL testing upon enrollment, and if viremic (VL>40 copies/ml) they underwent *pol* genotyping. Bioinformatic analyses were done with SQUAT (Delong et al; ARHR 2012;28:894) and Stanford HIV Sequence Database tools (http://hivdb.stanford.edu).

We compared outcomes among patients with (Prior-ART Group) and without (TDF-Only Group) prior non-TDF based first-line ART using Fisher exact and Wilcoxon Rank Sum tests. Our definition of treatment failure (VL>40 copies/ml) was compared to the WHO defined immunologic (persistent CD4 below 100 cells/µl, or fall to or below baseline) and virologic (VL>1,000 copies/ml) criteria.

To investigate codon patterns reported to be associate with K65R development, RT codons 64-66 from study participants were compared to those from AMPATH ART-naïve patients, and to those from treatment naïve and TDF-experienced patients across HIV-1 subtypes from the Stanford Database.

Figure 1: AMPATH Clinical Sites in Western Kenya



Red numbers, main clinics; green letters, satellite clinics; study site circled

Table 1: Characteristics of Study Participants

Variable		Total n=332	TDF only n=216	Prior ART n=116
Sex	Female	183/332, 55%	116/216, 54%	67/116, 58%
Age		41 (23, 82)	40 (23, 82)	44 (27, 66)
Missed Doses in Last Month	None	313/332, 94%	205/216, 95%	108/116, 93%
	Some (<50%)	19/332, 6%	11/216, 5%	8/116, 7%
Missed Doses in Last Week	None	325/332, 98%	212/216, 98%	113/116, 97%
	Some (<50%)	6/332, 2%	4/216, 2%	2/116, 2%
	Most (>50%)	1/332, 0%	0/216, 0%	1/116, 1%
Latest TDF Regimen	XTC,TDF,NVP	210/332, 63%	128/216, 59%	82/116, 71%
	XTC,TDF,EFV	122/332, 37%	88/216, 41%	34/116, 29%
CD4 Count		336 (5, 1043)	300 (5, 1035)	426 (44, 1043)
CD4 Percent		21 (1, 48)	20 (1, 48)	24 (7, 47)
Tuberculosis Ever		66/332, 21%	48/216, 22%	20/116, 18%
WHO Clinical Stage	1	81/332, 24%	59/216, 27%	22/116, 19%
	2	53/332, 16%	38/216, 18%	15/116, 13%
	3	143/332, 43%	86/216, 40%	57/116, 49%
	4	55/332, 17%	33/216, 15%	22/116, 19%
Opportunistic Infections		17/332, 5%	11/216, 5%	6/116, 5%
Total Months on ART		25 (6.9, 119)	20 (6.9, 51)	72 (20, 119)
Months on TDF treatment		21 (7, 68)	20 (7, 51)	24 (7, 68)
Months on non-TDF treatment		0 (0, 105)	n/a	47 (1, 105)
Viral Load		40 (40, 1351982)	40 (40, 1351982)	40, (40, 2192)
	VL≤40	274/332, 83%	166/216, 77%	108/116, 93%
	VL>40	58/332, 17%	50/216, 23%	8/116, 7%
	VL≥1000	299/332, 90%	184/216, 85%	115/116, 99%
	VL>1000	33/332, 10%	32/216, 15%	1/116, 1%
Reason for Switch to TDF	Lipodystrophy	n/a	n/a	81/116, 70%
	Anemia	n/a	n/a	11/116, 9%
	Neuropathy	n/a	n/a	7/116, 6%
	Anemia and Lipodystrophy	n/a	n/a	2/116, 2%

Footnote: Values are presented as median (range) for continuous variables and n/N, % for categorical variables. XTC refers to either FTC (emtricitabine) or 3TC (lamivudine). ART, Antiretroviral Treatment;TDF, Tenofovir; EFV, Efavirenz; NVP, Nevirapine; CD4 counts and % were missing for three participants

Figure 2: Treatment Failure by Study Group

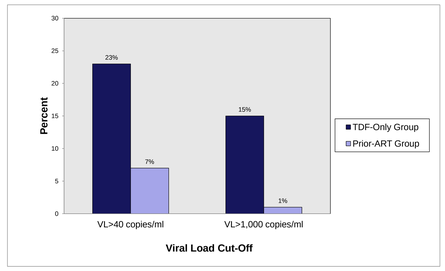


Table 2: Drug Resistance Mutations in the TDF-Only Group

Study ID	Subtype	Viral Load (copies/ml)	Last ART	NRTI	NNRTI
1	A	1,351,982	3TC,TDF,EFV	D67G, K70Q, M184V	K101P, K103S, E138Q
2	A	92,081	3TC,TDF,EFV	**K65R**, M184V	A98G, V179F, Y181C, G190A
3	A	337,468	3TC,TDF,EFV	**K65R**, K70T, M184I	V90I, L100I, K103N
4	A	31,683	3TC,TDF,EFV	**K65R**, M184I	V90I, K103N, M230L
5	A	287,657	3TC,TDF,EFV	**K65R**, V75M, Y115F, M184V	K103N, V108I, Y181C, G190A
6	A	297,459	3TC,TDF,NVP	A62AV, **K65R**, M184V	K101E, Y181C, G190A
7	A	3,825	3TC,TDF,NVP	M184V	G190A
8	A	87,827	3TC,TDF,NVP	K70E, Y115F, M184V	Y181C, H221Y
9	A	100,342	3TC,TDF,NVP	**K65R**, M184I	Y181C, M230L
10	A	3,516	3TC,TDF,NVP	A62AV, **K65R**, Y115FY, M184V	A98AG, K103KN, Y181C
11	A	8,247	3TC,TDF,NVP	**K65R**, M184MV	V90I, Y181G, G190A
12	A	89	3TC,TDF,NVP	None	K103N
13	A	41	3TC,TDF,NVP	None	None
14*	A	15,888	3TC,TDF,NVP	A62V, **K65R**, D67N, M184V, K219E	K101EK, E138AT, Y181C, G190A
15	A	4,983	3TC,TDF,NVP	M184I	K103N
16	A	47,280	3TC,TDF,NVP	None	None
17	A	150,649	3TC,TDF,NVP	**K65KR**, D67DG, M184MV	K101E, Y181CY, G190A
18	A	65,519	3TC,TDF,NVP	**K65R**, M184V	Y181C
19	A	1,541	3TC,TDF,NVP	**K65R**, M184I	V90I, V108I, Y181C
20	A	7,001	3TC,TDF,NVP	**K65R**	K103N, Y181C
21	A	9,476	3TC,TDF,NVP	**K65R**, Y115F, M184V	K103N, Y181C
22*	A	58,286	3TC,TDF,NVP	M41L, **K65KR**, L74V, M184V	K101E, Y181C, G190A
23	A	199,751	3TC,TDF,NVP	**K65R**, M184V	V108I, Y181C, H221Y
24	A	127,716	3TC,TDF,NVP	**K65R**, Y115FY	Y181C, H221Y
25	C	38,437	3TC,TDF,EFV	A62V, **K65R**, M184V	V106M, Y181C, G190S
26	C	60,147	3TC,TDF,NVP	**K65R**, Y115F, M184V	K101E, Y181C, G190A
27	C	5,585	3TC,TDF,NVP	A62AV, **K65KR**, K70EK, M184V	A98G, Y181C, G190A
28*	C	972	3TC,TDF,NVP	**K65KR**, D67DN, K70R, M184V, K219E	A98G, G190A
29	D	1,000,000	3TC,TDF,EFV	K70E, M184V	Y181C
30	D	7,968	3TC,TDF,NVP	**K65R**, M184V	K101P, K103N
31	D	939,458	3TC,TDF,NVP	**K65R**, M184V	K101E, Y181CFGV, G190AG
32	D	153	3TC,TDF,NVP	None	V106A
33*	AD	7,592	3TC,TDF,NVP	**K65R**, M184V, K219EK	K103N, Y181C
34	AD	45	3TC,TDF,NVP	None	None
35	AD	12,652	3TC,TDF,NVP	None	None

Footnote: Table sorted according to subtype, then last ART regimen and lastly viral load.
*Participants with K65R in whom thymidine analogue mutations (TAMs) were also found
Bolded mutations highlight K65R and K65KR mixtures.

Figure 3: Effect of Mutations on Predicted Drug Resistance

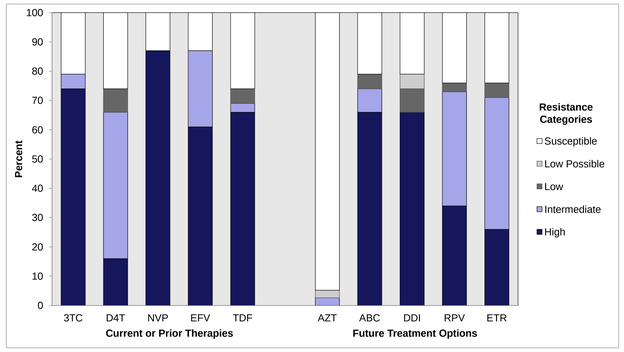
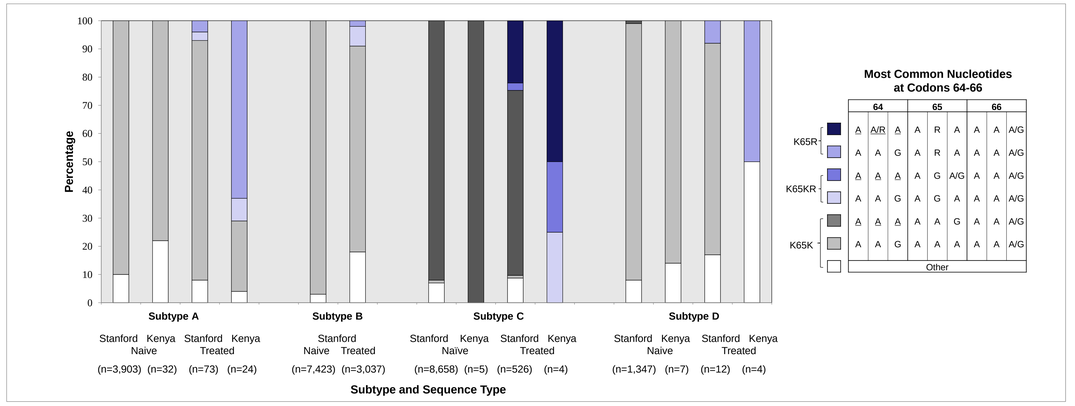


Figure 4: Codon Analysis of ART-Naïve and TDF-Treated Patients with HIV-1 Subtypes A, B, C and D



III. Results

A total of 332 patients were enrolled. Their demographics, clinical and laboratory characteristics can be found in Table 1.

Seventeen percent had detectable VL and 10% had VL> 1,000 copies/ml (WHO cutoff). VL was significantly higher in the TDF-Only Group than in the Prior-ART Group according to both the VL>40 copies/ml threshold (23% versus 7%; p<.001) and the VL>1,000 copies/ml threshold (15% versus 1%; p<.0001). (Figure 2)

TDF-Only Patients had lower CD4 values (p<.001) and higher WHO stage (p=.03). Treatment failure was not associated in either group with age, time on treatment or use of NVP-based regimens.

In 35 available genotypes from the TDF-Only Group, subtypes were diverse, including subtypes A (69%), C (11%), D (11%) and AD recombinants (9%).

RT resistance mutations were present in 89% of TDF-Only patients failing therapy, 89% to NNRTIs, 83% to NRTIs and 83% to both classes. A full list of mutations detected in patients failing therapy can be found in Table 2.

The K65R mutation occurred in 69% (24/35) of TDF-Only patients with sequences across subtypes: 17/24 (71%) patients with subtype A, 4/4 (100%) with subtype C, 2/4 (50%) with subtype D and 1/3 (33%) with AD recombinants (p=.197).

Patients with K65R had lower CD4 count (p=.019) and CD4 percent (p=.006), worse WHO clinical stage (79% versus 18% stage 3-4, p=.004), and a greater number of RT resistance mutations (100% versus 27% with 3 or more mutations, p=.001).

Only 4 of the 24 (17%) patients with K65R had any IAS-USA defined thymidine analogue mutations (TAMs; * in Table 2).

Predicted drug susceptibilities to current regimens and future drug options are shown in Figure 3. High or intermediate resistance to current or prior therapies was seen for 3TC in 29/35 (83%), FTC in 29/35 (83%), D4T in 24/35 (69%), TDF in 25/35 (71%), EFV in 31/35 (86%), NVP in 31/35 (89%) and both EFV and NVP in 31/35 (86%); and to future treatment options for AZT in 1/35 (3%), etravirine in 26/35 (74%) and rilpivirine in 26/35 (74%).

Examination of RT positions 64-66 from subtypes A and D infected ART-naïve persons from the same Kenya clinic (n=32), and ART-naïve and treated patients from the Stanford Database (n=3,903), demonstrated codon similarity to subtype B, without the poly-A chain that is thought to promote K65R in subtype C (Figure 4).

V. Summary and Conclusions

In this Kenyan cohort, the prior-ART group had low (7%) prevalence of detectable HIV VL, suggesting favorable outcomes upon switching from non-TDF to TDF-based first-line ART.

Higher virological failure was seen in the TDF-Only Group compared to the Prior-ART Group. This finding may be due to the Prior-ART group being healthier at the time TDF was started, or possibly due to lower K65R rates, though the latter could not be examined.

High K65R rates were observed in subtype A (71%) and D (50%), not explained by the poly-A template seen in subtype C at adjacent codons, suggesting alternative mechanisms such as limited access to virological and drug resistance monitoring.