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Implication of First-Line Antiretroviral Therapy Choice on Second-Line Options

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Background. Although there are a number of studies comparing the currently recommended preferred and alternative first-line (1L) antiretroviral therapy (ART) regimens on clinical outcomes, there are limited data examining the impact of 1L regimen choice and duration of virologic failure (VF) on accumulation of drug resistance mutations (DRM). The patterns of DRM from patients failing zidovudine (AZT)-containing versus tenofovir (TDF)-containing ART were assessed to evaluate the predicted susceptibility to second-line (2L) nucleoside reverse-transcriptase inhibitor (NRTI) backbone options in the context of an ongoing programmatic setting that uses viral load (VL) monitoring.

Methods. Paired samples from Nigerian ART patients who experienced VF and switched to 2L ART were retrospectively identified. For each sample, the human immunodeficiency virus (HIV)-1 polymerase gene was sequenced at 2 time points, and DRM was analyzed using Stanford University's HIVdb program.

Results. Sequences were generated for 191 patients. At time of 2L switch, 28.2% of patients on AZT-containing regimens developed resistance to TDF, whereas only 6.8% of patients on TDF-containing 1L had mutations compromising susceptibility to AZT. In a stratified evaluation, patients with 0–6 months between tested VL samples had no difference in proportion compromised to 2L, whereas those with >6 months between samples had a statistically significant difference in proportion with compromised 2L NRTI. In multivariate analyses, patients on 1L AZT had 9.90 times higher odds of having a compromised 2L NRTI option than patients on 1L TDF.

Conclusions. In the context of constrained resources, where VL monitoring is limited, we present further evidence to support use of TDF as the preferred 1L NRTI because it allows for preservation of the recommended 2L NRTI option.

Keywords. antiretroviral therapy; drug resistance; tenofovir; viral load monitoring; zidovudine.

The World Health Organization (WHO) recommends a simplified approach for choosing first-line (1L) and second-line (2L) antiretroviral therapy (ART) for treatment of human immunodeficiency virus (HIV)-1 in adults in resource-limited settings (RLS) [1, 2]. The preferred regimen is tenofovir (TDF) + lamivudine (3TC) or emtricitabine (FTC) + efavirenz (EFV). In situations where TDF+3TC/FTC+EFV is unavailable or contraindicated, zidovudine (AZT) + 3TC + nevirapine (NVP) is considered the alternative. For patients failing 1L, the WHO recommends a simplified approach to 2L nucleoside reverse-transcriptase inhibitor (NRTI) options: if AZT (or d4T) + 3TC was used in 1L ART, then TDF+FTC/3TC is the preferred NRTI backbone for 2L; alternatively, if TDF+3TC/FTC was used in 1L, then AZT+3TC is preferred for 2L. The 2L recommendation is based on the expectation that the previously unused NRTI backbone will have preserved activity. However, when detection of failure is delayed, which can occur when viral load (VL) monitoring is performed only every 12 months, as currently recommend in WHO guidelines [2], the accumulation of drug resistance mutations (DRM) may result in compromise of the 2L NRTI backbone [3–6].

Although the use of TDF-containing 1L regimens is increasing, many clinics continue to retain or newly initiate patients on AZT [7]. Although there are various studies comparing the preferred and alternative regimens on clinical outcomes [8–14], there are limited data regarding 1L regimen choice and duration of virologic failure (VF) on the accumulation of mutations, particularly in resource-constrained settings that have access to VL monitoring [15]. Although many patients retain sensitivity to TDF after failing AZT, some studies have found that patients continued on failing AZT-containing regimens for long periods, largely

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in situations where VL monitoring is unavailable, accumulate multiple thymidine-analog-associated mutations (TAMs) [3–6], compromising susceptibility to 2L NRTI options. However, if the order of NRTI drugs had been reversed or the timing between VL tests shortened, the recommended 2L NRTI would potentially remain a viable option. In the context of currently recommended ART regimen and monitoring protocols, additional research to determine the best sequence of drugs is needed.

To date, there are also limited data comparing the 2 currently recommended 1L regimens on the impact of accumulated DRM on subsequent 2L outcomes. In the Harvard/APIN PEPFAR Program in Nigeria, because of the concerns about potential lower efficacy of NRTIs due to accumulated mutations and the limited access to drug resistance testing, the clinical advisory staff, composed of experts from both Nigeria and the United States, recommended using 3 NRTI drugs (AZT+TDF+FTC/3TC) in the 2L regimen with the rationale that the regimen would provide expanded coverage. The practice of providing 3 NRTIs continued until approximately 2013, during which patients that were receiving 3 NRTIs were switched to 2, per WHO recommendations.

To understand the impact of retaining patients on failing regimens by 1L NRTI in the context of the recommended VL monitoring schedules, we evaluated the rate of drug resistance mutation accumulation in patients failing 1L. We also examined the subsequent response to 2L ART, with the limitation that the majority of patients were given a 2L regimen that contained 3 NRTIs. We examined DRM in paired samples from Nigerian ART patients from 3 different hospitals enrolled in a large-scale HIV care program based in Nigeria that were failing a 1L regimen. The study not only aimed to broaden our understanding of DRM in RLS, but also critical gaps in our knowledge of the implications of NRTI sequencing on 2L outcomes in RLS.

METHODS

Study Population

This study used samples from patients that received ART at 3 large tertiary treatment centers affiliated with the Harvard/AIDS Prevention Initiative in Nigeria, Ltd./Gte. (APIN) Centers for Disease Control and Prevention (CDC)-funded PEPFAR Program in Nigeria [16, 17]. The 3 sites include the Nigerian Institute of Medical Research (NIMR), Jos University Teaching Hospital (JUTH), and University College Hospital in Ibadan (UCH). All patients received treatment according to national and WHO guidelines at the time they were receiving treatment [1, 18–21]. Typically, 1L regimens contained 2 NRTIs and 1 non-NRTI (NNRTI), and 2L regimens were protease inhibitor (PI)-based, containing either lopinavir boosted with ritonavir (LPV/r) or atazanavir boosted with ritonavir (ATV/r) plus a combination of NRTIs. As aforementioned, due to concerns regarding lack of access to resistance data and about impact of mutations on susceptibility to any NRTIs used for 2L, the programmatic practice was to use 3 NRTIs with a PI for 2L to allow for expanded coverage; starting in 2013, patients on 2L were given 2 NRTIs with a PI.

Using electronic patient data collected at the sites between the years 2005–2013 [22], a cohort of ART-naive adult patients who initiated either AZT+3TC or TDF+3TC/FTC along with NVP or EFV for 1L and subsequently met WHO VF criteria (2 consecutive VL measurements ≥1000 copies/mL after at least 6 months on 1L ART) was retrospectively identified. Exclusion criteria included the following: (1) achieved VF, despite adequate adherence, but were never switched to 2L or resuppressed on 1L; (2) switched to 2L, but did not have at least 12 months retention post-2L switch; and (3) AZT/TDF substitutions prior to the time of 2L switch.

Ethics

Participants provided written informed consent before enrollment in the Harvard/APIN PEPFAR Program. The study protocols were approved by national and local research ethics committees, including the Institutional Review Boards at the Harvard T. H. Chan School of Public Health (Harvard Chan), NIMR, JUTH, and UCH. The protocol was also approved by the National Health Research Ethics Committee (NHREC) in Nigeria.

Laboratory Methods

From the start of the Harvard/APIN PEPFAR program in 2004, blood samples were drawn at baseline (ie, ART initiation), 3 months, and every 6 months thereafter unless clinical indications suggested an earlier draw. Starting in 2010, the program dropped the practice of VL testing at 3 months, and by 2014, programmatic and national guidelines shifted the recommendations to VL testing at months 6, 12, and then every 12 months thereafter. For each sample, standardized tests were performed to monitor $CD4^+$ T-cell counts, VL, hematology, and chemistry values, as previously described [23].

Drug resistance mutation genotypes were generated on the first available specimen after VF (S1: first of 2 samples with VL ≥1000 copies/mL) and on samples taken closest to the time of 2L switch (S2 sample). Drug resistance mutation assays for the S1 samples were conducted at the 3 laboratories in Nigeria using the ViroSeq HIV-1 Genotyping System 2.0 Assay (Abbot, Chicago, IL) or the American Type Culture Collection HIV-1 Drug Resistance Genotyping Kit (CDC, Atlanta, GA). Protease and reverse-transcriptase sequencing for the S2 samples were conducted at Harvard Chan using adapted in-house standardized primers [24]. All sequence data were edited and aligned with reference sequences from the Los Alamos HIV Sequence Database [25] using CLUSTAL X [26]. Bootstrapped neighbor-joining trees were generated for subtype determination using NJ Plot [27]. Mutation profiles and drug susceptibility were evaluated using Stanford University's HIVdb program, which follows International Antiviral Society-USA recommendations [28–30].

Statistical Methods

Patient characteristics were examined using univariate methods and were compared between those on AZT- versus TDF-containing 1L regimen using bivariate methods, which included *t* tests and Wilcoxon rank-sum tests for continuous variables and χ^2 or Fisher's exact tests for categorical variables, as relevant. Accumulation of DRM between S1 and S2 were calculated as a rate per month.

To evaluate the impact that remaining on a failing 1L regimen has on 2L susceptibility, a genotype susceptibility score (GSS) was calculated for each patient at both S1 and S2. The GSS was calculated based on the drug resistance report extracted from the Stanford HIVdb. Each antiretroviral (ARV) drug was assigned a score as follows: 1.00 for susceptible, 0.75 for potential low-level resistance, 0.50 for low-level resistance, 0.25 for intermediate resistance, and 0.0 for high-level resistance [31]. The GSS was the sum of all scores for each ARV included in the 2L regimen.

In preliminary data analyses, we found that the majority of patients in this study cohort were switched to 2L regimen that included TDF+AZT+3TC/FTC plus a PI (ie, LPV/r or ATV/r), as opposed to either TDF+3TC/FTC+PI or AZT+3TC+PI, as recommended in the WHO guidelines. Therefore, we also computed a GSS score as if only TDF+FTC with a PI or AZT+3TC with a PI was used (GSS_{rec}). To evaluate how the time between S1 and S2 impacted accumulation of mutations, we calculated a rate of change in GSS_{rec} between S1 and S2 by dividing difference in GSS_{rec} at S2 and S1 by time between S1 and S2. Rate of change of GSS_{rec} between S1 and S2 by 1L NRTI was compared using the Wilcoxon rank-sum test.

We calculated numbers of patients that were compromised to any drug in the recommended 2L regimen at the time of switch; patients with intermediate or high-level resistance on the Stanford HIVdb scale were coded as resistant to 2L. The prevalence of DRM at each time point, the percentage of patients with compromised 2L, and GSS for patients on TDF versus those on AZT for 1L was evaluated using the χ^2 or Fisher's exact test, as relevant. A multiple logistic regression model using backwards elimination was developed to assess the predictors of being compromised to the recommended 2L NRTI option. All variables significant at the $P = .20$ level in bivariate logistic regressions were considered for inclusion in the final model: we also retained all variables that served as effect modifiers. To examine impact of DRM on 2L VL outcomes, we evaluated the association between GSS (computed using prescribed 2L regimen) at S2 and VL outcomes at month 12 postswitch to 2L using the Fisher's exact test.

RESULTS

Cohort Characteristics

The study cohort contained 191 patients who were on 1L ART in the Harvard/APIN PEPFAR program and switched to 2L ART after confirmation of VF. Of those patients, 46.1% were on a TDF-containing 1L regimen and 53.9% were on an AZT-containing 1L regimen (Table 1). The median age for the cohort was 33 years and 71.7% of the patients were female. A majority of patients had at least a secondary level education, engaged in income-generating occupations, and were married. Of the 191 patients, 40.8% were infected with CRF02_AG and 35.1% were infected with subtype G or G-prime. There were some statistically significant baseline differences between those on TDF- versus AZT-containing 1L: a slightly higher percentage of patients on AZT versus TDF were in income-generating occupations versus non-income-generating occupations; baseline VL counts were higher in the TDF versus AZT group; and median baseline $CD4^+$ cell counts were slightly lower in the TDF group compared with the AZT group.

Drug Resistance Mutations at Time of First Virologic Failure (S1)

The median time from ART initiation (AI) to first detectable VL (S1) was 12.2 months, with a slightly longer time from AI to S1 in the AZT group compared with the TDF group ($P = .001$) (Table 1). Overall, 54.5% of the 178 patients that had a VL value before S1 achieved viral suppression before S1, with the AZT group having a higher percentage compared with the TDF group ($P = .005$). At S1, 165 patients (86.4%) had at least 1 NRTI mutation, with M184I/V being the most common of the NRTI mutations and K65R being the next most common (Table 2).

Accumulation of Mutations (S1 to S2)

The median time from S1 to S2 was 12.0 months, with the median time being statistically significantly shorter in those on TDF compared with those on AZT ($P = .0003$) (Table 1) for 1L. From S1 to S2, the percentage of patients with ≥3 TAMs rose from 8.7% to 35.0%, and the percentage of patients with both a TAM-1 and TAM-2 mutation increased from 5.8% to 20.4% (Table 2). Between S1 and S2, new DRM accumulated with a median rate of 0.08 DRM per month (Figure 1A). The median rate for patients on TDF-containing 1L was 0.05 DRM/month, and for those on AZT-containing 1L it was 0.10 ($P = .08$) (Figure 1B).

Predicted Drug Susceptibility by Drug Exposure

At S1, we found no difference in 2L NRTI susceptibility in patients that had 1L TDF versus AZT (Figure 2A). However, at S2, patients who received 1L TDF were less likely to be compromised to AZT as compared to those who received 1L AZT were to be compromised to TDF (Figure 2A). To adjust for the different time between S1 and S2 in patients on 1L TDF versus AZT, we stratified the time and found that patients who only had 0–6 months between S1 and S2 had no difference in proportion that were compromised to the 2L NRTI backbone by 1L NRTI ($P = .64$), whereas those with >6 months between S1 and S2 had a statistically significant difference in proportion with compromised 2L NRTI (Figure 2B).

In a multivariate analysis, we found that time between S1 and S2 no longer remained a significant predictor of having a

Table 1. Demographic and Clinical Characteristics of Patients Who Experienced Virologic Failure

Characteristic	Total	1L NRTI		*P* Value
		TDF	AZT	
N	191	88 (46.1)	103 (53.9)	
1L NNRTI, n (%)				.06
NVP	162 (84.8)	70 (79.6)	92 (89.3)	
EFV	29 (15.2)	18 (20.5)	11 (10.7)	
Female sex, n (%)	137 (71.7)	63 (71.6)	74 (71.8)	.97
Median age at baseline, years (IQR)	33 (28–39)	34 (28–39)	32 (27–40)	.58
Site, n (%)				.13
JUTH	121 (63.4)	51 (58.0)	70 (68.0)	
NIMR	65 (34.0)	36 (40.9)	29 (28.2)	
UCH	5 (2.6)	1 (1.1)	4 (3.9)	
ART Initiation Year, n (%)				.003
2005	27 (14.1)	11 (12.5)	16 (15.5)	
2006	39 (20.4)	12 (13.6)	27 (26.2)	
2007	32 (16.7)	9 (10.2)	23 (22.3)	
2008	37 (19.4)	20 (22.7)	17 (16.5)	
2009	31 (16.2)	18 (20.4)	13 (12.6)	
≥2010	25 (13.1)	18 (20.4)	7 (6.8)	
Education, n (%)				.57
None	17 (8.9)	8 (9.1)	9 (8.7)	
Primary	45 (23.6)	24 (27.3)	21 (20.4)	
Secondary	63 (33.0)	28 (31.8)	35 (34.0)	
Tertiary	60 (31.4)	27 (30.7)	33 (32.0)	
Marital Status, n (%)				.74
Single	52 (27.2)	25 (28.4)	27 (26.2)	
Married	102 (53.4)	46 (52.3)	56 (54.4)	
Divorced/Separated	12 (6.3)	4 (4.6)	8 (7.8)	
Widowed	25 (13.1)	13 (14.8)	12 (11.6)	
Occupation Type, n (%)				.04
Nonincome-generating	55 (28.8)	32 (36.4)	23 (22.3)	
Income-generating	136 (71.2)	56 (63.6)	80 (77.7)	
Baseline WHO Stage, n (%)				.002
1	43 (22.5)	10 (11.4)	33 (32.0)	
2	61 (31.9)	30 (34.1)	31 (30.1)	
3	68 (35.6)	39 (44.3)	29 (28.2)	
4	13 (6.8)	8 (9.1)	5 (4.9)	
Baseline Log VL, copies/mL, n (%)				.03
≤5.0	87 (45.6)	32 (36.4)	55 (53.4)	
>5.0	81 (42.4)	46 (52.3)	35 (34.0)	
Median (IQR)	5.0 (4.5–5.4)	5.2 (4.8–5.5)	4.8 (4.3–5.3)	.0004
Baseline $CD4^+$ cell count, cells/mm^3, n (%)				.02
≤100	114 (59.7)	61 (69.3)	53 (51.5)	
>100	77 (40.3)	27 (30.7)	50 (48.5)	
Median (IQR)	90 (44–147)	75 (38–114)	101 (54–163)	.01
HIV-1 Pol Subtype, n (%)				.48
A	11 (5.8)	4 (4.6)	7 (6.8)	
C	3 (1.6)	0 (0.0)	3 (2.9)	
D	3 (1.6)	1 (1.1)	2 (1.9)	
F	1 (0.5)	0 (0.0)	1 (1.0)	
G/G′	67 (35.1)	35 (39.8)	32 (31.1)	
CRF02_AG	78 (40.8)	36 (40.9)	42 (40.8)	
CRF06_cpx	8 (4.2)	5 (5.7)	3 (2.9)	
Indeterminate	20 (10.5)	7 (8.0)	13 (12.6)	
Median time on 1L ART, months (IQR)				
ART Initiation to S1	12.2 (9.6–18.2)	11.3 (8.4–14.1)	13.2 (11.2–19.1)	.001
S1 to S2	12.0 (5.8–17.9)	8.8 (5.0–16.0)	14.6 (8.0–21.5)	.0003
S2 to Switch to 2L	1.6 (0.9–3.3)	1.3 (0.9–3.0)	1.8 (0.9–3.4)	.39

Table 1. Continued

Characteristic	Total	1L NRTI		*P* Value
		TDF	AZT	
Achieved VL Suppression before S1, n (%)	97 (54.5)	35 (43.2)	62 (63.9)	.005
Median (IQR) CD4$^+$ count, copies/mL				
At S1	172 (111–274)	135 (80–211)	215 (148–308)	<.0001
At S2	134 (83–237)	116 (62–161)	187 (99–318)	<.0001
Median (IQR) VL at S1, copies/mL				
S1	4.5 (3.9–4.9)	4.6 (4.1–5.1)	4.4 (3.8–4.9)	.04
S2	4.6 (4.1–5.1)	4.7 (4.2–5.2)	4.5 (3.9–4.8)	.005
2L ART Regimen, n (%)				<.001
TDF+AZT+3TC/FTC+LPV/r	118 (61.8)	44 (50)	74 (71.8)	
TDF+AZT+3TC+ATV/r	50 (26.2)	25 (28.4)	25 (24.3)	
Other	23 (12.0)	19 (21.6)	4 (3.9)	
GSS				
At S1	3.00 (2.00–3.00)	2.00 (2.00–2.62)	3.00 (2.50–3.00)	<.0001
At S2	2.00 (1.50–2.50)	2.0 (2.00–2.00)	1.75 (1.25–3.00)	.88
GSS_{rec}				
At S1	2.00 (2.00–2.00)	2.00 (2.00–2.00)	2.00 (2.00–2.00)	.61
At S2	2.00 (1.50–2.00)	2.00 (2.00–2.00)	1.50 (1.25–2.00)	<.0001
Achieved VL suppression at 12 months on 2L	119 (73.9)	52 (74.3)	67 (73.6)	.93

Abbreviations: 1L, first-line; 2L, second-line; 3TC, lamivudine; ART, antiretroviral therapy; ATV/r, atazanavir boosted with ritonavir; AZT, zidovudine; EFV, efavirenz; FTC, emtricitabine; GSS, genotype susceptibility score; HIV, human immunodeficiency virus; IQR, interquartile range; JUTH, Jos University Teaching Hospital; LPV/r, lopinavir boosted with ritonavir; NIMR, Nigerian Institute of Medical Research; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; NVP, nevirapine; TDF, tenofovir; UCH, University College Hospital in Ibadan; VL, viral load; WHO, World Health Organization.

compromised 2L (Table 3). It is interesting to note that when controlling for 1L NNRTI, treatment site, AI year, occupation type, WHO stage, baseline CD4$^+$ cell count, and VL, time from AI to S1, time from S1 to S2, and having a suppressed VL before S1, 1L NRTI remained a significant predictor of having a compromised 2L, where those on AZT had 9.90 times higher odds of having a compromised 2L NRTI option than those patients who had TDF for their 1L NRTI.

The median GSS_{rec} at S1 was 2.0 (interquartile range [IQR], 2.0–2.0), with the median being 2.0 (IQR, 2.0–2.0) for those on 1L TDF and 2.0 (IQR, 2.0–2.0) for those on 1L AZT (Table 1). The GSS_{rec} at S2 was 2.0 (IQR, 1.5–2.0), with median value being 2.0 (IQR, 2.0–2.0) for those on 1L TDF and 1.50 (IQR, 1.25–2.00) for those on 1L AZT. The median rate of decrease in GSS_{rec} per year for the cohort was 0.0 (IQR, 0.0–0.34), with the decrease being 0.0 GSS_{rec} drug/year (IQR, 0.0–0.0) for those on 1L TDF and 0.15 GSS_{rec} drug/year (IQR, 0.00–0.45) for those on 1L AZT.

Viral Load Outcomes on Second-Line Regimen

In total, 168 (88.0%) of the included patients were switched to a 2L regimen that contained TDF+AZT+3TC/FTC, along with a boosted PI (Table 1). Of the 161 (84.3%) patients with 12-month VL data postswitch, 119 (73.9%) had an undetectable VL (UDVL); a higher percentage of patients on TDF+AZT+3TC/FTC, regardless of PI, had a UDVL at month 12 compared with those other combinations of 2L ART (75.9% vs 60.0%; $P = .13$).

At S1, when data were combined for all patients, GSS ranged from 1.00 to 4.00, with a median of 3.00 (IQR, 2.00–3.00) (Table 1). The median score for those on 1L TDF was 2.00 (IQR, 2.00–2.62), and for those on AZT was 3.00 (IQR, 2.50–3.00). At S2, the median score was 2.00 (IQR, 1.50–2.50): the median was 2.00 (IQR, 2.00–2.00) for the TDF group and 1.75 (IQR, 1.25–3.00) for the AZT group. The median rate of decrease of GSS from S1 to S2 for those on TDF was 0.00 (IQR, 0.00–0.45) drug/year as compared to 0.50 (IQR, 0.00–1.00) drug/year for the AZT group.

At S2, the majority of patients (66.0%) had a GSS ≥2, with 85.2% of those on TDF with GSS ≥2 versus 49.5% of those on AZT for 1L NRTI ($P < .001$). Although there was a slightly larger percentage of patients with higher GSS with UDVL at month 12 postswitch to 2L ART, where 75.5% of patients with GSS ≥2 had an UDVL compared with 70.9% of patients with GSS <2, we were not able to show a statistically significant association between GSS and 2L VL outcome ($P = .57$; data not shown).

DISCUSSION

This study provides important data on the accumulation of mutations and development of cross-resistance to 2L NRTI backbone options for patients on a failing 1L ART regimen. Similar to other studies, we found that duration on ART is associated with number of DRM and development of cross-resistance [32–38]. Specifically, we found that patients failing on 1L TDF had fewer deleterious NRTI mutations and were more likely to be susceptible to the 2L NRTI option (ie, AZT) than patients that received 1L AZT. Because we sampled 2 serially collected samples per patient, we could evaluate the time at which the cross-resistance developed. Although we found no difference in risk if the time between the S1 and S2 measurements

Table 2. Frequency of Major NRTI Mutations by 1L NRTI at Two Measured Time Points

Mutation Type and Number	S1 All (n = 191)	S1 TDF (n = 88)	S1 AZT (n = 103)	S1 *P* Value	S2 All (n = 191)	S2 TDF (n = 88)	S2 AZT (n = 103)	S2 *P* Value
Number of NRTI Mutations				<.001				<.001
0	26 (13.6)	10 (11.4)	16 (15.5)		5 (2.6)	1 (1.1)	4 (3.9)	
1	69 (36.1)	11 (12.5)	58 (56.3)		35 (18.3)	5 (5.7)	30 (29.1)	
2	51 (26.7)	39 (44.3)	12 (11.7)		42 (22.0)	26 (29.6)	16 (15.5)	
≥3	45 (23.6)	28 (31.8)	17 (16.5)		109 (57.1)	56 (63.6)	53 (51.5)	
Any TAM Mutations				.02				<.001
0	145 (75.9)	70 (80.0)	75 (72.8)		95 (49.7)	61 (69.3)	34 (33.0)	
1	26 (13.6)	15 (17.1)	11 (10.7)		36 (18.9)	20 (22.7)	16 (15.5)	
2	9 (4.7)	1 (1.1)	8 (7.8)		20 (10.5)	3 (3.4)	17 (16.5)	
≥3	11 (5.8)	2 (2.3)	9 (8.7)		40 (20.9)	4 (4.6)	36 (35.0)	
TAM I Mutations								
M41L	7 (3.7)	0 (0.0)	7 (6.8)	.02	29 (15.2)	0 (0.0)	29 (28.2)	<.001
L210W	3 (1.6)	0 (0.0)	3 (2.9)	.25	13 (6.8)	0 (0.0)	13 (12.6)	<.001
T215Y	10 (5.2)	1 (1.1)	9 (8.7)	.02	32 (16.8)	1 (1.1)	31 (30.1)	<.001
Multiple TAM I Mutations				.02				<.001
0	178 (93.2)	87 (98.9)	91 (88.4)		150 (78.5)	87 (98.9)	63 (61.2)	
1	8 (4.2)	1 (1.1)	7 (6.8)		17 (8.9)	1 (1.1)	16 (15.5)	
2	3 (1.6)	0 (0.0)	3 (2.9)		15 (7.9)	0 (0.0)	15 (14.6)	
≥3	2 (1.1)	0 (0.0)	2 (1.9)		9 (4.7)	0 (0.0)	9 (8.7)	
TAM II Mutations								
D67N	19 (10.0)	6 (6.8)	13 (12.6)	.23	36 (18.9)	9 (10.2)	27 (26.2)	.005
K70R	15 (7.9)	2 (2.3)	13 (12.6)	.01	37 (19.4)	4 (4.6)	33 (32.0)	<.001
T215F	9 (4.7)	2 (2.3)	7 (6.8)	.14	24 (12.6)	2 (2.3)	22 (21.4)	<.001
K219E	15 (7.9)	12 (13.6)	3 (2.9)	.007	33 (17.3)	22 (25.0)	11 (10.7)	.01
K219Q	8 (4.2)	2 (2.3)	6 (5.8)	.22	19 (10.0)	2 (2.3)	17 (16.5)	.001
Multiple TAM II Mutations				.37				.002
0	152 (79.6)	71 (80.7)	81 (78.6)		115 (60.2)	62 (70.5)	53 (51.5)	
1	26 (13.6)	14 (15.9)	12 (11.7)		39 (20.4)	19 (21.6)	20 (19.4)	
2	5 (2.6)	1 (1.1)	4 (3.9)		11 (5.8)	3 (3.4)	8 (7.8)	
≥3	8 (4.2)	2 (2.3)	6 (5.8)		26 (13.6)	4 (4.5)	22 (21.4)	
TAM I and TAM II Mutations	6 (3.1)	0 (0.0)	6 (5.8)	.03	21 (11.0)	0 (0.0)	21 (20.4)	<.001
Other NRTI Mutations								
M184I/V	158 (82.7)	72 (81.8)	86 (83.5)	.76	185 (96.9)	87 (98.9)	98 (95.2)	.22
K65R	46 (24.1)	46 (52.3)	0 (0.0)	<.001	57 (29.8)	57 (64.8)	0 (0.0)	<.001
Y115F	16 (8.4)	16 (18.2)	0 (0.0)	<.001	31 (16.2)	31 (35.2)	0 (0.0)	<.001
K70E	16 (8.4)	16 (18.2)	0 (0.0)	<.001	18 (9.4)	18 (20.5)	0 (0.0)	<.001
A62V	8 (4.2)	6 (6.8)	2 (1.9)	.15	12 (6.3)	12 (13.6)	0 (0.0)	<.000
V75I	3 (1.6)	3 (3.4)	0 (0.0)	.10	6 (3.1)	4 (4.6)	2 (1.9)	.42
F77L	1 (0.5)	1 (1.1)	0 (0.0)	.46	1 (0.5)	1 (1.1)	0 (0.0)	.46
F116Y	1 (0.5)	1 (1.1)	0 (0.0)	.46	1 (0.5)	1 (1.1)	0 (0.0)	.46
Q151M	1 (0.5)	1 (1.1)	0 (0.0)	.46	1 (0.5)	1 (1.1)	0 (0.0)	.46
L74V	0 (0.0)	0 (0.0)	0 (0.0)		1 (0.5)	1 (1.1)	0 (0.0)	.46

Abbreviations: 1L, first-line; AZT, zidovudine; NRTI, nucleoside reverse-transcriptase inhibitor; TAM, thymidine-analog-associated mutation; TDF, tenofovir.

was between 0 and 6 months, patients who had greater than 6 months between S1 and S2 had marked difference between the TDF and AZT groups with regards to proportion with a predicted compromised 2L NRTI backbone option.

Our finding is supported by data from a meta-analysis examining optimal VL monitoring frequency, where cohorts that received infrequent VL monitoring were more likely to have DRM compared with those that received frequent VL monitoring [39].

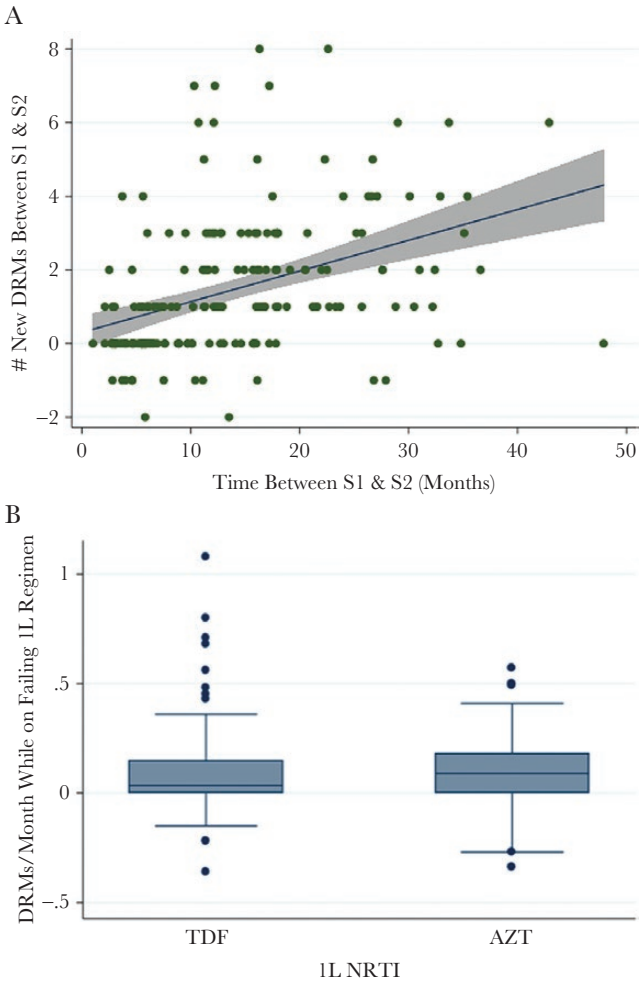

Figure 1. Drug resistance mutations (DRMs) accumulated between S1 and S2 time points. (A) Number of new mutations from S1 to S2; (B) rate of accumulation of mutations between S1 and S2 by first-line (1L) nucleoside reverse-transcriptase inhibitor (NRTI). Abbreviations: AZT, zidovudine; TDF, tenofovir.

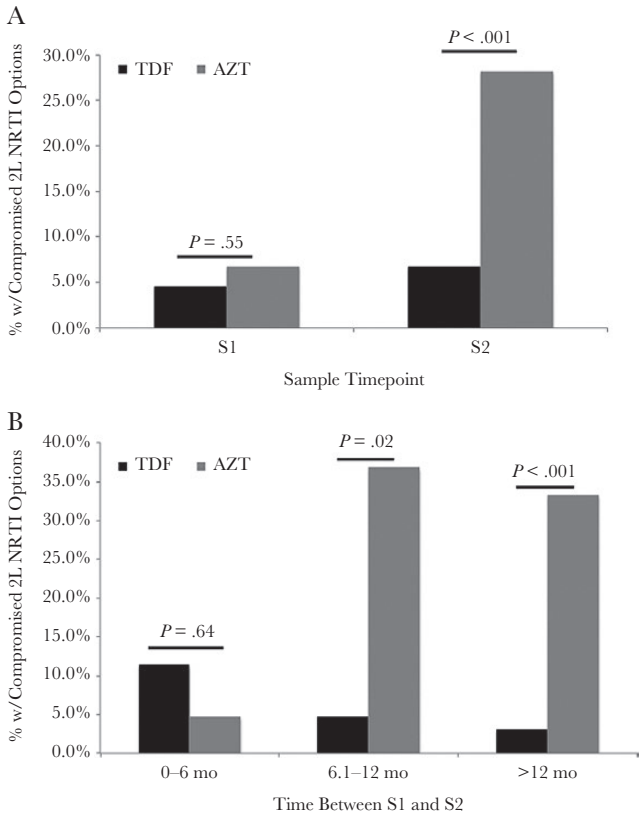

Figure 2. Percentage of patients with a compromised second-line (2L) nucleoside reverse-transcriptase inhibitor (NRTI) option by first-line NRTI: (A) by sample time point and (B) by time between S1 and S2 sample. Abbreviations: AZT, zidovudine; TDF, tenofovir.

Patients included in this study who enrolled in the earlier years of the Harvard/APIN PEPFAR program received VL testing at months 3, 6, and 12 postinitiation of ART and every 6 months thereafter; patients enrolled from 2010 onwards received VL testing at months 6, 12, and then every 6 months thereafter. Our data reveal that in a real-world programmatic setting, patients could be on a failing regimen for an extended period of time due to delays in sample processing, backlogs of samples, poor sample quality, and unavailability of test kits, despite establishment of protocols aimed at following prevailing guidelines.

As anticipated, given that the majority of patients in the patient population received 3 NRTIs (TDF+AZT+3TC/FTC) as part of their 2L, we found that 73.9% of the patients with VL data had an UDVL after 12 months on 2L. If 2L NRTIs were prescribed according to the WHO guideline recommendations, we anticipate that patients with GSS <2 near the time of switch might have been less likely to be suppressed at 12 months postswitch versus those with GSS ≥2. Our findings are consistent with those of the EARNEST study that showed the addition of 2 NRTIs, with little or no predicted efficacy due to resistance, had an effect equivalent to using raltegravir and possibly propagated the activity via viral fitness mechanisms rather than through direct drug activity [40].

There are a number of strengths of this study. To date, few studies in RLS have examined mutations in serial samples from the same patient to evaluate accumulation of mutations. As such, the study design allowed for quantification of mutation rates within individual patients rather than across populations. Another strength is that because TDF and AZT were concurrently in use in the treatment population, we were able to evaluate both 1L NRTI backbones concurrently in the same programmatic setting. In addition, because the program had already been utilizing VL testing for monitoring ART outcomes, we were well suited to conduct this study and had significant data spanning over a decade of treatment.

This study has a few limitations, the first being its retrospective design based on an observational cohort, which meant we were restricted to available samples, had minimal control over sample quality and sample testing schedules, and were biased towards patients that had regular VL availability. Another limitation was that we did not have data on potential pretreatment mutations;

Table 3. Predictors of Having Compromised 2L NRTI Option at S2 (Time Closest to Switch to 2L)

Predictor	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
1L NRTI AZT (vs TDF)	5.36 (2.11–13.62)	9.90 (2.58–37.99)
1L NNRTI EFV (vs NVP)	1.90 (0.76–4.75)	3.46 (0.84–14.15)
Female sex (vs male)	0.52 (0.24–1.11)	
Age, years	1.00 (0.96–1.05)	
NIMR Site (vs JUTH and UCH)	0.20 (0.07–0.60)	0.08 (0.01–0.69)
ART Initiation Year (vs 2005)		
2006	1.38 (0.44–4.32)	0.94 (0.21–4.21)
2007	0.98 (0.28–3.37)	0.55 (0.09–3.44)
2008	0.68 (0.19–2.39)	0. 95 (0.15–6.06)
2009	0.24 (0.04–1.32)	0.89 (0.08–9.63)
2010	0.48 (0.11–2.16)	5.38 (0.28–101.61)
Education (vs none)		
Primary	2.95 (0.33–25.95)	
Secondary	3.76 (0.45–31.24)	
Tertiary	4.43 (0.01–0.47)	
Marital Status (vs single)		
Married	1.87 (0.70–4.99)	
Divorced/separated	5.48 (1.31–22.85)	
Widowed	1.46 (0.06–0.31)	
Income-generating occupation (vs nonincome generating)	1.46 (0.62–3.44)	1.17 (0.39–3.48)
WHO Stage 3/4 at baseline (vs stage 1/2)	1.16 (0.56–2.42)	2.16 (0.76–6.17)
Baseline $CD4^+$ cell count >100 cells/mm^3 (vs ≤100)	1.13 (0.54–2.39)	.0.99 (0.36–2.74)
Baseline Log VL >5.0 cp/mL (vs ≤5.0)	0.72 (0.32–1.61)	1.16 (0.42–3.178)
Time from ART initiation to S1, months	0.98 (0.94–1.03)	0.96 (0.88–1.04)
Time from S1 to S2, months	1.04 (1.00–1.08)	1.00 (0.94–1.07)
Suppressed VL before S1	1.24 (0.58–2.65)	1.48 (0.42–5.17)

Abbreviations: 1L, first-line; 2L, second-line; ART, antiretroviral therapy; AZT, zidovudine; CI, confidence interval; EFV, efavirenz; JUTH, Jos University Teaching Hospital; NIMR, Nigerian Institute of Medical Research; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; NVP, nevirapine; OR, odds ratio; TDF, tenofovir; UCH, University College Hospital in Ibadan; VL, viral load; WHO, World Health Organization.

however, this evaluation only included patients that were previously ARV-naive with presumed low levels of DRM. Finally, because the majority of our patients received TDF+AZT+3TC/FTC in their 2L, we were unable to evaluate the impact of the mutations on 2L outcomes when the standard regimens were to be used; however, the data did reveal that use of 3 NRTIs in 2L resulted in better outcomes than if only 2 NRTIs were used.

CONCLUSIONS

Our data indicate that using TDF versus AZT in 1L ART is preferable because there is a higher likelihood of retained susceptibility to the recommended 2L NRTI option (ie, AZT), particularly in the context of a RLS where VL testing is either unavailable or where there are delays between testing and accessing of results by a clinician. Our data also support the notion that the differences in 2L NRTI susceptibility are minimized if time between VL tests is shortened [4, 5], particularly once a high VL is detected. Our findings have important implications for 1L ART regimen and monitoring recommendations, specifically the order in which NRTI drugs are used in 1L and 2L regimens, but also with regard to potential changes in VL monitoring if AZT is the prescribed regimen.

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Potential conflicts of interest. All authors: No reported conflicts of interest.

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