Published in final edited form as:

AIDS Res Hum Retroviruses. 2018 February ; 34(2): 228–233. doi:10.1089/AID.2017.0198.

Distinct Pattern of Thymidine Analogue Mutations with K65R in Patients Failing Tenofovir-Based Antiretroviral Therapy

Beth Chaplin¹, Godwin Imade², Chika Onwuamah³, Georgina Odaibo⁴, Rosemary Audu³, Jonathan Okpokwu², David Olaleye⁴, Seema Meloni¹, Holly Rawizza^{1,5}, Mohammad Muazu², Adesola Z. Musa³, Jay Samuel⁶, Oche Agbaji², Oliver Ezechi³, Emmanuel Idigbe³, Phyllis J. Kanki¹

¹Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health, Boston, Massachusetts.

²Jos University Teaching Hospital, Jos, Nigeria.

³Nigerian Institute of Medical Research, Lagos, Nigeria.

⁴University College Hospital, Ibadan, Nigeria.

⁵Brigham and Women's Hospital, Boston, Massachusetts.

⁶AIDS Prevention Initiative Nigeria, Ltd./Gte., Abuja, Nigeria.

Abstract

Historically, in HIV patients, the K65R mutation and thymidine analogue mutations (TAMs) have been reported to rarely coexist. We retrospectively reviewed genotype data from paired samples in a cohort of HIV-1-infected Nigerian patients failing first-line antiretroviral therapies containing zidovudine (AZT) or tenofovir (TDF). Samples for each patient were taken at initial confirmed virological failure 1000 copies/ml (S1) and then at the latest available sample with viral load 1000 copies/ml before switch to second line (S2). Among 103 patients failing AZT, 19 (18.4%) had TAM-1s, 29 (28.2%) TAM-2s, and 21 (20.4%) mixed TAMs by S2. In contrast, in the 87 patients failing TDF, drug resistance mutations at S2 included K65R in 56 (64.4%), TAM-1s in 1 (1.1%), and TAM-2s in 25 patients (28.7%). Interestingly, 30.4% of patients with K65R in our study developed TAMs. These were exclusively K219E ± D67N and were not predicted to confer a resistance cost to future AZT-containing regimens.

Keywords

HIV drug resistance; thymidine analogue mutations; Nigeria; tenofovir; zidovudine; HIV

Address correspondence to: Phyllis J. Kanki, DVM, DSc, Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health, 651 Huntington Avenue, FXB 404, Boston, MA 02115, pkanki@hsph.harvard.edu.

Sequence Data

The sequences reported in this study were deposited in GenBank under accession numbers [MF622552](#) to [MF622689](#) and from [MF622692](#) to [MF622933](#).

Author Disclosure Statement

No competing financial interests exist.

The scaleup of antiretroviral therapy (ART) for HIV-positive patients in sub-Saharan Africa has made it important to assess differences in the development of drug resistance mutations (DRMs) in the context of patient populations infected with nonsubtype B HIV-1 variants and real-world constraints of resource-limited settings. Here we report our findings on a cohort of Nigerian patients failing first-line (1L) antiretroviral (ARV) therapies containing zidovudine (AZT) or tenofovir (TDF). The primary goal of this retrospective study was to compare the potential impact of 1L regimen choice on future susceptibility to second-line (2L) ART options. The collection and testing of paired samples from each patient also gave us the unique opportunity to closely examine the evolution of DRMs, including thymidine analogue mutations (TAMs) and K65R.

As described previously,¹ patients from three treatment centers affiliated with the Harvard/AIDS Prevention Initiative in Nigeria, Ltd./Gte. (APIN) CDC-funded PEPFAR Program: Jos University Teaching Hospital, Jos, Nigeria (JUTH); Nigerian Institute of Medical Research, Lagos, Nigeria (NIMR); and, University College Hospital in Ibadan, Ibadan, Nigeria (UCH), were selected and their stored samples were examined. As per standard of care, these patients had blood samples drawn at ART initiation, and then every 6 months thereafter, following a schedule of monitoring for CD4⁺ T cell counts, viral load, hematology, and chemistry values. A long-term outcomes study in this population assessed virological failure in 30,792 patients and found an overall rate of 96.5 cases per 1,000 person-years (PY).² Criteria for patient selection for this study included patients naïve to ARVs at time of entry; 1L regimen of AZT or TDF+lamivudine (3TC) or emtricitabine (FTC)+nevirapine (NVP) or efavirenz (EFV); confirmed virological failure to 1L regimen on or after 6 months on treatment; and subsequent switch to 2L antiretroviral therapy (ART). The study was limited to patients that remained exclusively on one nucleoside reverse transcriptase inhibitor (NRTI) backbone, AZT or TDF, throughout the length of their 1L regimen. Plasma samples were sequenced at initial confirmed virological failure with viral load ≥ 1,000 copies/ml (S1), and then at the latest available time point with viral load < 1,000 copies/ml before switch to 2L regimen (S2).

Protease and reverse transcriptase were sequenced either by ViroSeq HIV-1 Genotyping System 2.0 Assay (Abbott, Chicago, IL) or by the HIV-1 Drug Resistance Genotyping Kit (ATCC, Manassas, VA) at the laboratory in which they were collected, for S1. Sequencing for the S2 samples was conducted at the Harvard T.H. Chan School of Public Health using adapted in-house standardized primers.³ For both, sequence data were edited and analyzed for quality control, before the Stanford HIVdb program was used to generate lists of mutations and polymorphisms. Sequences were aligned in Clustal X and compared with reference sequences obtained from the Los Alamos repository using neighbor-joining trees to classify them by subtype.

The 190 patients included in the study have been characterized previously for the impact on 2L regimen choices; this subanalysis differed in cohort composition and analysis methods.¹ In brief, 87 patients were on a TDF-containing regimen and 103 on AZT (Table 1). The median time on 1L regimen from S1 to S2 for the TDF group was 8.9 months (IQR: 5.2–16.1) and that for the AZT group was 14.6 months (IQR: 8.0–21.5; $p = .0003$). The most

common subtypes identified were CRF02_AG (41.1%), G (34.7%), A (5.8%), and CRF06_cpx (4.2%), consistent with other data from Nigeria.⁴

Owing to the low genetic barrier for resistance to EFV and NVP, non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance has been previously found to develop quickly.^{5,6} The most common mutations detected were Y181C, K103N, G190A, A98G, K101E, V108I, H221Y, M230L, and P225H. By virological failure at S1, 91.6% of patients had 1 NNRTI, and 66.8% of patients had 2 NNRTIs, and at S2, respectively, 97.4% and 79.5%. In this population, Y181C was more common in NVP-exposed patients, whereas K103N, V108I, and P225H were more common in EFV-exposed patients ($p < .001$).

As expected, M184I/V was the most common NRTI mutation, present in 82.6% of patients at S1 and 96.8% at S2 (Fig. 1). There was no significant difference in the prevalence of M184I/V at S1 or S2 between patients failing FTC or 3TC, or between patients with TDF or AZT exposure.

In patients failing an AZT-containing regimen, TAMs were the most common DRMs after M184I/V. Based on significant previous research of DRMs, TAMs have been described to associate into one of two distinct pathways: TAM-1 (M41L, L210W, T215Y) or TAM-2 (D67N, K70R, T215F, K219E/Q).⁷ In studies based on U.S. and European populations, the TAM-1 pathway is described as being more common, and it confers a greater negative impact on virological response to TDF-containing regimens than does the Type 2 pathway.⁸ In this study, 6/28 (21.4%) of S1 patients failing AZT-based therapy with 1 TAM had exclusively TAM-1, 16/28 (57.1%) had TAM-2, and 6/28 (21.4%) had mixed TAMs. At S2 sampling, 19/69 (27.5%) had TAM-1, 29/69 (42.0%) had TAM-2, and 21/69 (30.4%) had mixed. The higher prevalence of TAM-2 mutations is in accordance with some^{9,10} but not all¹¹ studies from nonsubtype B settings. As expected, patients failing AZT-based therapy did not develop mutations K65R, K70E, L74V, or Y115F, and only rarely were DRMs from the Q151M complex seen.

In patients failing TDF-based therapy, the predominant NRTI DRM aside from M184I/V was K65R, which was present in 45/87 (51.7%) S1 and 56/87 (64.4%) S2 patients. Also common were associated DRMs K70E and Y115F.

K65R emerged more often by S2 in patients on a regimen in which the 1L TDF regimen contained 3TC (16/19, 84.2%) than FTC (14/32, 43.8%) ($p = .005$). Based on drug availability to the program, 3TC was prescribed more frequently in patients enrolling in the year 2008 and later, so a logistic regression model was used to determine whether year of ART start or time on treatment could explain the differences in the emergence of K65R. When controlling for both, K65R was still less likely to appear in patients on FTC than on 3TC ($p = .004$).

Despite lack of exposure to AZT or other ARVs that are known to be associated with the development of TAMs, certain TAMs were also present in these patients, and distinct patterns were seen. When K65R was present, the only TAMs detected were K219E ± D67N in 7/45 (15.6%) S1 samples and in 17/56 (30.4%) S2 samples. In viruses lacking K65R, the TAMs observed included a broader array of primarily TAM-2, including K219E, D67N,

K70R, T215F, and K219Q, and in one patient with TAM-1, T215Y. TAMs were observed in 23.8% of samples at S1 and 29.0% of samples at S2, in those without K65R (Fig. 2).

In comparing the observed versus expected frequencies of individual mutations, K219E was 26% more likely to appear with K65R than expected in both S1 and S2 samples in patients on TDF. This was not true of other TAMs present in patients on 1L TDF, such as T215Y, D67N, K70R, T215F, or K219Q.

Our finding of a relatively high frequency of K219E in patients with K65R was surprising. Previous research has shown that TAMs and K65R rarely coexist on the same genome.^{12,13} However, other groups have reported similar results: Etiebet *et al.*, looking at Nigerian patients failing 1L regimen, found that 2 of 23 patients with TDF exposure had K219E, in a population with predominantly subtypes G and CRF02_AG.⁹ Dinesha *et al.* examined 167 Indian patients with immunological failure on TDF-based 1L regimen, all with subtype C infection, and found TAMs, some coexisting with K65R.¹⁰

In a reanalysis by Gregson *et al.*, looking at 20 studies, 115 of 712 (16.2%) patients failing 1L TDF in sub-Saharan Africa had at least one TAM.¹⁴ These findings come from a mixture of countries and subtypes, including a small number from the same clinics described in this article, so direct comparisons are not possible; however, the predominant TAMs seemed to be D67N, K219E, and M41L. The authors speculated that the likely reason for most of the TAMs in these TDF-treated patients could be previous undisclosed ART use. We cannot discount this possibility in our cohort; however, ARV dispense information was recorded on a monthly basis and a close examination of patient histories did not show evidence of previous ART use. We further investigated the possibility of prior prevention of mother-to-child transmission (PMTCT) interventions that could have included AZT, as 71.6% of the study cohort was female. Available antenatal and delivery records were examined, but it was possible for a patient to have received PMTCT at a different clinic, or to have unrecorded use. However, three males in the study exhibited the K65R, K219E $\pm$ D67N pattern. It is also worth noting that in the cases of the patients harboring TAMs that coexist with K65R, the distinct patterns seen in the TDF-treated patients differ markedly from those in AZT-treated patients.

Without performing single-genome sequencing, we cannot verify that these TAMs coexist on the same virus with K65R, but in terms of the potential clinical importance of the patterns, they are all present in the same patient at levels detectable by population sequencing (~20%). In the 56 patients with K65R present at S2, none of the TAMs (K219E $\pm$ D67N) were predicted to cause resistance to the AZT component of a potential 2L regimen. However, in the 31 patients with no K65R present at S2, 6 had intermediate or high-level resistance to AZT: 4 were caused by TAM-2 DRMs, 1 by T215Y, and 1 by Q151M-complex mutations Q151M, A62V, V75I, F77L, F116Y (Fig. 3).

Finally, we computed the rate of acquisition of new TAMs between S1 and S2. We calculated the rate in each patient as 1 divided by the time from S1 to S2, for patients who did not have the DRM at S1 but developed it by S2, or 0 for those who did not have the DRM at S1 and did not acquire it by S2. For all TAMs, with the exception of K219E, D67N,

and K70R, there was no acquisition from S1 to S2 in patients on TDF, whereas the mean acquisition rates of individual TAMs in the patients on AZT varied from 0.005 mutations per month to 0.019 mutations per month. In contrast, K219E developed significantly more quickly in patients on TDF (mean of 0.017 mutations per month) than in patients on AZT (0.006 mutations per month).

In conclusion, contrary to widespread thought that TAMs do not develop in the presence of K65R, our study found that nearly one-third of patients with K65R developed TAM-2s. Although this does not appear to confer a significant resistance cost to future 2L ART options, we have provided new data that build on previous studies, suggesting that some widely accepted dogma might not hold true in all settings. Our study design that involved the retrospective evaluation of paired samples was an important means of examining the patterns of accumulated mutations in a representative subset of patients who failed ART. Our findings emphasize the need for ongoing research on the unique patterns of DRMs seen among patients with differing HIV-1 subtypes globally.

Acknowledgments

This work was funded, in part, by the U.S. Department of Health and Human Services, Health Resources and Services Administration [U51HA02522], and the Centers for Disease Control and Prevention (CDC) through cooperative agreements [PS001058, U2G GH000770]. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the respective funding agencies.

References

1. Meloni ST, Onwuamah CK, Agbaji O, et al.: Implications of first-line antiretroviral therapy choice on second-line options. *Open Forum Infectious Diseases* 2017;ofx233: 10.1093/ofid/ofx233.
2. Meloni ST, Chang CA, Eisen G, et al.: Long-term outcomes on antiretroviral therapy in a large scale-up program in Nigeria. *PLoS One* 2016;11:e0164030. [PubMed: 27764094]
3. Zhou Z, Wagar N, DeVos JR, et al.: Optimization of a low cost and broadly sensitive genotyping assay for HIV-1 drug resistance surveillance and monitoring in resource-limited settings. *PLoS One* 2011;6:e28184. [PubMed: 22132237]
4. Chaplin B, Eisen G, Idoko J, et al.: Impact of HIV type 1 subtype on drug resistance mutations in Nigerian patients failing first-line therapy. *AIDS Res Hum Retroviruses* 2011;27:71–80. [PubMed: 20964479]
5. Boender TD, Kityo CM, Boerma RS, et al.: Accumulation of HIV-1 drug resistance after continued virological failure on first-line ART in adults and children in sub-Saharan Africa. *J Antimicrob Chemother* 2016;71:2918–2927. [PubMed: 27342546]
6. Cozzi-Lepri A, Paredes R, Phillips AN, et al.: The rate of accumulation of nonnucleoside reverse transcriptase inhibitor (NNRTI) resistance in patients kept on a virologically failing regimen containing an NNRTI*. *HIV Med* 2012;13:62–72. [PubMed: 21848790]
7. Menéndez-Arias L: Molecular basis of human immunodeficiency virus drug resistance: An update. *Antiviral Res* 2010;85:210–231. [PubMed: 19616029]
8. Cozzi-Lepri A, Ruiz L, Loveday C, et al.: Thymidine analogue mutation profiles: Factors associated with acquiring specific profiles and their impact on the virological response to therapy. *Antivir Ther* 2005;10:791–802. [PubMed: 16312176]
9. Etiebet MA, Shepherd J, Nowak RG, et al.: Tenofovir-based regimens associated with less drug resistance in HIV-1-infected Nigerians failing first-line antiretroviral therapy. *AIDS* 2013;27:553–561. [PubMed: 23079810]
10. Dinesha TR, Gomathi S, Boobalan J, et al.: Genotypic HIV-1 drug resistance among patients failing tenofovir-based first-line HAART in South India. *AIDS Res Hum Retroviruses* 2016;32:1234–1236. [PubMed: 27334566]

11. Crawford KW, Waskabi S, Kibuuka H, et al.: East meets west: A description of HIV-1 drug resistance mutation patterns of patients failing first line therapy in PEPFAR clinics from Uganda and Nigeria. *AIDS Res Hum Retroviruses* 2014;30:796–799. [PubMed: 24798614]
12. von Wyl V, Yerly S, Böni J, et al.: Factors associated with the emergence of K65R in patients with HIV-1 infection treated with combination antiretroviral therapy containing tenofovir. *Clin Infect Dis* 2008 46:1299–1309. [PubMed: 18444871]
13. Parikh UM, Barnas DC, Faruki H, et al.: Antagonism between the HIV-1 reverse-transcriptase mutation K65R and thymidine-analogue mutations at the genomic level. *J Infect Dis* 2006;194:651–660. [PubMed: 16897664]
14. Gregson J, Kaleebu P, Marconi VC, et al.: Occult HIV-1 drug resistance to thymidine analogues following failure of first-line tenofovir combined with a cytosine analogue and nevirapine or efavirenz in sub Saharan Africa: A retrospective multi-centre cohort study. *Lancet Infect Dis* 2017; 17:296–304. [PubMed: 27914856]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

FIG. 1.

Frequency of NRTI DRMs in patients on AZT-based 1L regimen or TDF-based 1L regimen. *Solid colors* show the percentage accumulation of each mutation by the S1 sampling, and *dotted colors* show the continued accumulation between S1 and S2. Mutations that occur at significantly different frequencies between the two groups at S2, after correcting for multiple comparisons, are indicated with an *asterisks*. DRMs, drug resistance mutations.

FIG. 2.

TAM distribution by NRTI backbone and associated DRMs. **(A)** TDF or **(B)** AZT. The cumulative frequencies of individual TAMs are shown in the bar charts to the right: **i.** Frequency of individual TAMs coexisting with K65R at S2, in patients on 1L TDF. **ii.** Frequency of individual TAMs in samples with no K65R at S2, in patients on 1L TDF. **iii.** Frequency of individual TAMs at S2, in patients on 1L AZT. TAM, thymidine analogue mutation.

FIG. 3.

Predicted response to AZT or TDF component of 2L regimen, as evaluated by the Stanford University HIVdb program. Resistance is graded on a 5-level scale: high-level resistance, intermediate resistance, low-level resistance, potential low-level resistance, or susceptible. **(A)** TDF-based 1L regimen: predicted response to AZT. **(B)** AZT-based 1L regimen: predicted response to TDF. All predicted resistance to AZT in TDF patients comes from samples in which K65R is not present. In the samples for which K65R is present, (shown with *diagonal marks*), patients are all susceptible to AZT.

Table 1.

CHARACTERISTICS OF FIRST-LINE ANTIRETROVIRAL THERAPY PATIENTS AT BASELINE AND VIROLOGICAL FAILURE

	All patients (n = 190)	TDF exposure (n = 87)	AZT exposure (n = 103)	p
Sex				
Male	54 (28.4)	25 (28.7)	29 (28.2)	.93
Female	136 (71.6)	62 (71.3)	74 (71.8)	
Site				
JUTH	121 (63.7)	51 (58.6)	70 (68.0)	.159
NIMR	64 (33.7)	35 (40.2)	29 (28.2)	
UCH	5 (2.6)	1 (1.2)	4 (3.9)	
NNRTI exposure ^a				
Primarily NVP	157 (86.7)	67 (81.7)	90 (90.9)	.07
Primarily EFV	24 (13.3)	15 (18.3)	9 (9.1)	
Cytosine analogue exposure ^b				
Primarily 3TC	122 (79.2)	19 (37.3)	103 (100.0)	.000
Primarily FTC	32 (20.8)	32 (62.8)	0	
HIV-1 subtype				
CRF02_AG	78 (41.1)	36 (41.4)	42 (40.8)	.29
G	66 (34.7)	34 (39.1)	32 (31.1)	
Indeterminate/other	27 (14.2)	8 (9.2)	19 (18.5)	
A	11 (5.8)	4 (4.6)	7 (6.8)	
CRF06_cpx	8 (4.2)	5 (5.8)	3 (2.9)	
Median time on 1L ART, months (IQR)				
ART initiation to S1	12.2 (9.6–18.2)	11.3 (8.3–14.3)	13.2 (11.2–19.1)	.001
S1 to S2	12.0 (5.8–17.9)	8.9 (5.2–16.1)	14.6 (8.0–21.5)	.0004

As described previously,¹ patients from three treatment centers affiliated with the Harvard/AIDS Prevention Initiative in Nigeria, Ltd./Gte. (APIN) CDC-funded PEPFAR Program: Jos University Teaching Hospital, Jos, Nigeria (JUTH); Nigerian Institute of Medical Research, Lagos, Nigeria (NIMR); and, University College Hospital in Ibadan, Ibadan, Nigeria (UCH), were selected and their stored samples were examined. As per standard of care, these patients had blood samples drawn at ART initiation, and then every 6 months thereafter, following a schedule of monitoring for CD4⁺ T cell counts, viral load, hematology, and chemistry values. Criteria for patient selection included patients naïve to ARVs at time of entry; 1L regimen of AZT or TDF+lamivudine (3TC) or entricitabine (FTC)+nevirapine (NVP) or efavirenz (EFV); confirmed virological failure to 1L regimen on or after 6 months of treatment; and subsequent switch to 2L antiretroviral therapy (ART). The study was limited to patients who remained exclusively on one NRTI backbone, AZT or TDF, throughout the length of their 1L regimen. Plasma samples were sequenced at initial confirmed virological failure with viral load > 1000 copies/ml (S1), and then at the latest available time point with viral load > 1000 copies/ml before switch to 2L regimen (S2).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

^aClassified by primary NNRTI exposure; patients with mixed exposure to NNRTIs were excluded.
^bClassified by primary cytosine analogue exposure; patients with mixed exposure to FTC/3TC were excluded. ART, antiretroviral therapy; NNRTI, non-nucleoside reverse transcriptase inhibitor.