Published in final edited form as:
Antivir Ther. 2013 ; 18(7): 915–920. doi:10.3851/IMP2652.

Resistance to tenofovir-based regimens during treatment failure of subtype C HIV-1 in South Africa

Christopher J Hoffmann[1,2,*], **Johanna Ledwaba**[3], **Jin-fen Li**[4], **Victoria Johnston**[5], **Gillian Hunt**[3], **Katherine L Fielding**[5], **Richard E Chaisson**[1], **Gavin J Churchyard**[2,5,6], **Alison D Grant**[5], **Jeffrey A Johnson**[4], **Salome Charalambous**[2], and **Lynn Morris**[3]

[1]Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

[2]Aurum Institute, Johannesburg, South Africa

[3]Centre for HIV and STIs, National Institute for Communicable Diseases of the National Health Laboratory Services, Johannesburg, South Africa

[4]HIV Drug Resistance and Diagnostics Development Unit, Division of HIV/AIDS Prevention, Centers for Disease Control and Prevention, Atlanta, USA

[5]London School of Hygiene and Tropical Medicine, London, United Kingdom

[6]School of Public Health, University of the Witwatersrand, Johannesburg, South Africa

Abstract

Background—Tenofovir disoproxil fumarate (TDF) is increasingly available for patients infected with subtype C HIV-1. This subtype is reported to develop the principle TDF resistance mutation in the HIV reverse transcriptase, K65R, with greater propensity than other subtypes. We sought to describe K65R development during TDF use in a cohort of patients infected with subtype C HIV.

Methods—Using a prospectively followed cohort with 6 monthly HIV RNA assays, we identified virologic failure (defined as an HIV RNA >1000 c/mL) during treatment that included TDF. Residual serum, stored at the time of the HIV RNA assay, was used for consensus sequencing and allele-specific PCR. We assessed prevalence of resistance at failure during TDF-containing treatment and associated factors.

Results—Among 1,682 patients on a TDF-containing regimen, 270 developed failure of which 40 were assessed for resistance. By sequencing, the K65R was identified in 5 (12%), major NNRTI mutations in 24 (57%), and the M184V/I in 12 (28%) patients. The K65R was associated with lower HIV RNA at failure (HIV RNA $\log_{10}$ 3.3 versus 4.2 c/mL) and prior stavudine exposure. An additional 5 patients had minority K65R populations identified by allele-specific PCR.

Conclusions—These data suggest that the K65R prevalence at virologic failure is moderately higher in our subtype C population than some non-subtype C HIV cohorts. However, we did not

*Corresponding author choffmann@jhmi.edu.

Disclosures: All authors report no conflicts of interest to disclose.

find that the K65R was highly selected in HIV-1 subtype C infected patients with up to 6 months of failure of a TDF-containing regimen.

Introduction

Tenofovir disoproxil fumarate (TDF) is recommended as a preferred first-line antiretroviral therapy (ART) agent by the World Health Organization (1). TDF is potent, allows once-a-day dosing, and is well tolerated; however, one of the concerns with its use is the potential for regional variation in the development of HIV drug resistance. Subtype C HIV-1, the subtype that predominates in southern Africa and India, has been reported to develop the principle TDF-associated drug resistance mutation, K65R in the HIV reverse transcriptase, more rapidly than other subtypes during *in vitro* selection (2–4). In addition, there may be a higher percentage of minority K65R quasi-species among ART-naïve individuals infected with subtype C compared to subtype B (5). Furthermore, use of stavudine (d4T) selects for the K65R mutation in a higher proportion of patients with subtype C HIV than may have been expected from the subtype B experience (6–9).

However, knowledge related to K65R development during treatment with TDF remains limited in settings in which subtype C HIV predominate and results differ widely (10;11). A recent study conducted in Durban, South Africa reported that among 33 patients with virologic failure while receiving a TDF-containing ART regimen, 23 (70%) had the K65R mutation, suggesting an unusually high prevalence of TDF resistance at treatment failure (11). This is in contrast to a report on K65R prevalence at virologic failure from an international collaboration of mostly European cohorts in which the proportion of subtype C HIV with the K65R was 16.2% (10) and a study from multiple African sites in which the prevalence was 27.7% (12). In addition, it is unclear whether there are specific risk factors for the K65R development during treatment failure while on TDF. In order to further examine the selection of K65R in a subtype C population, we described HIV drug resistance, including the prevalence of minority K65R, K70E, and M184V species, and associations with resistance, including prior receipt of either d4T or zidovudine (AZT), among patients at the time of virological failure on a TDF-containing ART regimen.

Methods

Patients in this study were part of a multisite workplace and community-based HIV programme managed by a single organization in South Africa (13;14). ART eligibility was based on CD4 count and WHO clinical stage criteria with a CD4 count threshold of <200 cells/mm^3 for much of the study period. HIV RNA and CD4 count were determined before ART initiation and every six months thereafter. Residual plasma, when available, was stored at −80°C. The first-line regimen was a combination of either AZT or d4T, lamivudine, and either efavirenz or nevirapine until mid-2007 in the workplace clinics, and mid-2010 in the community clinics. Subsequent to 2007 and 2010, there was a switch to TDF in the workplace and community programmes, respectively. Inclusion criteria for this study were as follows: patients were aged >17 years old and received a TDF-containing ART regimen along with a non-nucleoside reverse transcriptase inhibitor (NNRTI) greater than six months before the study closer date of March 2011, and had at least one HIV RNA value of <400

c/mL while on ART. Patients who underwent a single drug substitution from another NRTI to TDF, while remaining on an NNRTI, were included if the most recent HIV RNA prior to substitution to TDF was <400 c/mL; however, this HIV RNA assay could have occurred several months prior to the switch. Single drug substitutions generally occurred because of side effects, from either nucleoside reverse transcriptase inhibitor (NRTI) d4T or AZT. Follow-up time was censored after March 2011. All laboratory and treatment data were captured prospectively in a monitoring and evaluation database.

We defined treatment failure as an HIV RNA >1000 c/mL while on TDF with a prior HIV RNA <400 c/mL after initiating ART (either on TDF or just prior to substitution to TDF). All data were anonymised prior to analysis. Research conformed to the ethical principles set out in the Declaration of Helsinki. Ethical approval was provided by the University of the Witwatersrand, London School of Hygiene and Tropical Medicine, and Johns Hopkins University.

Laboratory

HIV RNA was assayed by polymerase chain reaction (PCR) (Amplicor HIV-1 Monitor Test, Roche Diagnostics, Nutley, New Jersey, USA) or branched chain DNA analysis (Bayer Versant, New York, USA). Genotyping was performed on a stored plasma sample available at the time of first viremia (HIV RNA >1000 c/mL) while on TDF using a validated in-house assay (15). Samples that failed to amplify were re-tested using a nested PCR to obtain a smaller fragment of 1,084 base pairs, as described elsewhere (16). Subtype C allele-specific PCR (AS-PCR) assays were used to detect low-level K65R, K70E, and M184V mutations. The delta cycle thresholds (ΔCT) and the mutation frequency cutoffs were 8.0 and ≥2% for K65R, 7.0 and ≥0.3% for K70E, and 8.5 and ≥0.5% for M184V (5).

Analysis

We compared characteristics at first ART initiation (on AZT, d4T, or TDF containing regimen) and at virologic failure on a TDF-containing regimen based on the first NRTI the patient had received (AZT, d4T, or TDF). We used the Kruskal-Wallis method, for continuous data, and chi-square test, for proportions. We also used the Kruskal-Wallis method to compare CD4 count, HIV RNA, and duration on TDF prior to failure among patients with and without stored plasma. We used non-parametric methods due to small sample sizes with sequencing data. We described the prevalence of mutations and 95% confidence intervals using the binomial exact method. We included the confidence interval of the prevalence of resistance mutations to provide a range of uncertainty for the true prevalence in our cohort, assuming no bias in which patients had samples available. We assessed for association between treatment site, duration on treatment, CD4 count, HIV RNA, prior NRTI exposure, sex, and age and presence of the K65R, M184V, or any major NNRTI mutation, based on the IAS-USA list of drug resistance mutations (17), using non-parametric methods; either the Wilcoxon rank sum test or Fisher’s exact test, as appropriate.

Results

A total of 1,682 patients met the inclusion criteria, of whom 843 (50%) were male; the median age was 37 years (interquartile range [IQR]: 32, 45), and the median CD4 count prior to ART initiation was 194 cells/mm^3 (IQR: 108, 328; Table 1). TDF was part of the initial regimen for 640 (38%) patients, whereas 206 (12%) were switched from AZT, and 836 (50%) were switched from d4T (Table 1). Initial NRTI groups differed by proportion male, age, and CD4 count at ART initiation (all $p < 0.05$). In addition, the median time on ART prior to switching to a TDF-containing regimen was longer for patients started on d4T (791 days) versus started on AZT (639 days; $p=0.005$).

Virologic failure was identified among 270 of the 1,682 patients (16%) while on TDF. The median CD4 count, HIV RNA, and time on TDF at virologic failure were similar across the three starting-NRTI groups (Table 1; all $p \geq 0.1$). The overall cohort median time on TDF at virologic failure was 152 days, CD4 count at failure was 199 cells/mm^3, and HIV RNA level was 4.3 $\log_{10}$ c/mL. Of the 270 patients with virologic failure, 44 (16%) had stored plasma from the date of first detection of virologic failure. We compared the CD4 count, HIV RNA, and duration on TDF at the time of failure between patients with stored plasma and those without stored plasma. Time on TDF at failure was longer for those with stored samples (261 days versus 140 days, $p<0.001$); whereas CD4 count and HIV RNA at failure were similar between patients with and without stored serum ($p>0.1$). The median time from the prior suppressed HIV RNA for patients with samples was 177 days (IQR: 124, 209).

Drug Resistance Mutations

Of the 44 patients with available samples from the time of virologic failure, HIV RNA could not be amplified from 4 due to low volume. The 40 patients with amplified HIV RNA were all infected with subtype C HIV-1; 22 (55%) of whom had drug resistance mutations detected by population sequencing (Table 2). NNRTI mutations were the most common (21; 52%), followed by the M184V/I mutation (11, 28%; 10 with M184V and 1 with M184I). Five (12%; 95% confidence interval: 4.1–27%) patients had the K65R mutation, four of whom also had the M184V mutation. No patients had the K70E by population sequencing.

AS-PCR was completed on 39 of the 40 samples. One sample had insufficient volume for AS-PCR; a sample that contained the K65R and M184V mutations by consensus sequencing. AS-PCR identified the presence of M184V in 10 patients (9 identified by sequencing and one additional patient). Four of the five samples that had the K65R mutation by sequencing also had the K65R mutation detected by AS-PCR (the fifth had insufficient volume for AS-PCR); in addition, five patients who were negative for the K65R by sequencing were positive by AS-PCR. Among individuals with positive AS-PCR for the K65R mutation and with negative consensus sequencing, the median ΔC_T value was 7.4 (IQR: 6.9, 7.4); close to the threshold representing 2% of quasi-species. No K70E mutations were detected by AS-PCR.

We evaluated for associations between the presence of the K65R, M184V, or major NNRTI mutations as identified through consensus sequencing. We identified an association between lower HIV RNA at virologic failure and the presence of resistance mutations (Table 3). This

was especially notable for patients with the K65R mutation; the HIV RNA IQR was 3.2–3.7 $\log_{10}$ c/mL when this mutation was present compared to 3.1–3.9 $\log_{10}$ c/mL when the M184V mutation was present and 3.2–4.5$\log_{10}$ c/mL when major NNRTI mutations were present. The five patients with the K65R by sequencing had all received d4T before switching to TDF. Among those with the K65R identified only with AS-PCR, two had received prior d4T, one prior AZT, and two had no history of ART prior to TDF initiation. There was no evidence for associations between HIV drug resistance and sex, age, HIV RNA prior to ART initiation,CD4 prior to ART initiation, or workplace versus community program (all p>0.1), although numbers were small leading to a very low power to detect an association.

Discussion

The K65R mutation was present among 12% (confidence interval of 4.1 – 27) of individuals at first detection of virologic failure of a TDF-containing regimen. This prevalence is similar to some prior studies based in clinical cohorts from subtype C or mixed subtype infections, in which the K65R mutations ranged from 0 to 27% and duration on a TDF-containing regimen ranged from 324 to 1000 days (8;10;12;18–21). Notably, our results are consistent with a modest increase in K65R associated with subtype C HIV, similar to the 16% prevalence reported in a multi-national analysis of subtype C HIV (10) and with overlapping confidence intervals with another study in Africa in which 27.7% of the 47 participants receiving TDF developed the K65R mutation (12). It is notable that our finding is markedly lower than the 70% reported from a study from Durban, South Africa (11) and predictions from some *in vitro* studies (2;3;5).

Differences in the prevalence of resistance identified in our study and from the Durban study may have arisen for a variety of reasons. Duration on ART at the time of failure is one possibility; however, this is an unlikely explanation as the difference in median time on ART between the studies was small (173 for the Durban study versus 152 days overall for this study and 261 days for those with sequencing) (11). It is plausible that participants who developed virologic failure in our study had stopped taking ART and thus did not maintain a predominant quasi-species that included resistance to TDF, lamivudine, or efavirenz. To further assess the prevalence of K65R with this scenario, we excluded patients without resistance mutations and only included the 22 patients with any mutations. This increased the K65R proportion to 5/22 (23%). This is higher than reports from clinical trials with subtype B (22–24) but still considerably lower than the Durban study. Another approach is to include the low-level K65R mutants detected by AS-PCR, making the assumption that these minority quasi-species were on the way to becoming dominant and were not detected by our consensus sequencing. However, this approach only leads to a prevalence of 25% (10/40). Another possibility could be the use of alternative resistance pathways that may have antagonism toward the K65R, such as TAMS and the K70E (25–27). However, only four patients developed TAMs and0 no K70E mutations were detected by either population sequencing or AS-PCR, making this an unlikely explanation. Finally, it is possible that a greater proportion of patients in the Durban study had previously received d4T; however, the authors reported that only 10 of the 35 patients had received d4T prior to TDF.

Several limitations need to be considered regarding our study. It is likely that some patients took little ART, leading to a high HIV RNA and no resistance mutations. However, we lacked reliable adherence data to assess this. Another limitation is the small number of samples available for resistance assays. We doubt bias influenced which patients had residual stored plasma as samples were missing either because insufficient specimen was sent to the laboratory for plasma storage following HIV RNA assay, samples were stored but were not able to be retrieved, or laboratory personnel failed to store specimen on a given day. The patients with and without stored residual plasma were also similar by CD4 count and HIV RNA, although time on TDF at the time of virologic failure was longer among those with sequencing. A longer duration on a regimen could lead to a higher proportion with resistance mutations. It is also possible that some patients had already developed virologic failure while on a d4T-based regimen and were not virologically suppressed at the time of TDF substitution. Where this occurred, some of the identified mutations, including K65R, may have developed during d4T exposure. It is unclear how this affected our results; however, if d4T did select for the K65R mutation, we may have overestimated the effect of TDF on K65R prevalence. Finally median duration on ART was relatively short. A longer time on ART may lead to an increase in K65R, especially if patients with virologic failure were maintained on a failing regimen for an extended period of time. Despite these limitations, we believe our analysis provides additional valuable information on K65R development in subtype C HIV.

In our cohort of patients with subtype C HIV who were receiving regular HIV RNA monitoring while on a TDF-containing ART, NNRTI and M184V mutations predominated while resistance to TDF occurred less frequently. Indeed, it appeared that prior d4T use was a risk for the K65R mutation in this cohort, possibly because the mutation was already being selected during prior d4T use. If prior enrichment is the case, it needs to be considered in selecting second-line agents or alternative first-line agents for individuals with d4T exposure in need of another agent. In the setting of prior d4T exposure, AZT may be a better option than TDF; however, studies are needed comparing second-line treatment options. For patients without prior ART exposure we did not identify any K65R mutations by population sequencing; while re-assuring that this mutation does not appear to develop rapidly, our small sample size and relatively short duration of failure requires circumspection. Our overall finding of a moderately higher prevalence of the K65R mutation among patients with subtype C HIV and virologic failure on a TDF-containing regimen adds to evidence for differences in the development of HIV drug resistance by HIV-1 genotype but is also re-assuring in that we did not find a markedly higher prevalence of the K65R that could have greater clinical importance.

Acknowledgments

Funders: C.J.H. was supported by National Institutes of Health AI083099; V.J. by a Wellcome Trust Fellowship; R.E.C. by National Institutes of Health AI5535901 and AI016137; and A.D.G. by a Global Health Trials (G1100689) and the Bill and Melinda Gates Foundation (OPP1034523).

Reference List

1. World Health Organization. Antiretroviral therapy for HIV infection in adults and adolescents. Austria: WHO; 2010.
2. Brenner BG, Oliveira M, Doualla-Bell F, Moisi DD, Ntemgwa M, Frankel F, et al. HIV-1 subtype C viruses rapidly develop K65R resistance to tenofovir in cell culture. AIDS. 2006 Jun 12; 20(9):F9–F13. [PubMed: 16816549]
3. Wainberg MA, Zaharatos GJ, Brenner BG. Development of antiretroviral drug resistance. N Engl J Med. 2011 Aug 18; 365(7):637–646. [PubMed: 21848464]
4. Invernizzi CF, Coutsinos D, Oliveira M, Moisi D, Brenner BG, Wainberg MA. Signature nucleotide polymorphisms at positions 64 and 65 in reverse transcriptase favor the selection of the K65R resistance mutation in HIV-1 subtype C. J Infect Dis. 2009 Oct 15; 200(8):1202–1206. [PubMed: 19764886]
5. Li JF, Lipscomb JT, Wei X, Martinson NA, Morris L, Heneine W, et al. Detection of low-level K65R variants in nucleoside reverse transcriptase inhibitor-naive chronic and acute HIV-1 subtype C infections. J Infect Dis. 2011 Mar 15; 203(6):798–802. [PubMed: 21257741]
6. Orrell C, Walensky RP, Losina E, Pitt J, Freedberg KA, Wood R. HIV type-1 clade C resistance genotypes in treatment-naive patients and after first virological failure in a large community antiretroviral therapy programme. Antivir Ther. 2009; 14(4):523–531. [PubMed: 19578237]
7. Hosseinipour MC, van Oosterhout JJ, Weigel R, Phiri S, Kamwendo D, Parkin N, et al. The public health approach to identify antiretroviral therapy failure: high-level nucleoside reverse transcriptase inhibitor resistance among Malawians failing first-line antiretroviral therapy. AIDS. 2009 May 4.
8. van Zyl GU, van der Merwe L, Claassen M, Zeier M, Preiser W. Antiretroviral resistance patterns and factors associated with resistance in adult patients failing NNRTI-based regimens in the Western Cape, South Africa. J Med Virol. 2011 Oct; 83(10):1764–1769. [PubMed: 21837793]
9. Pillay V, Pillay C, Kantor R, Venter F, Levin L, Morris L. HIV Type 1 Subtype C Drug Resistance among Pediatric and Adult South African Patients Failing Antiretroviral Therapy. AIDS Res Hum Retroviruses. 2008 Nov 11.24:1449–1453. [PubMed: 19000027]
10. Theys K, Vercauteren J, Snoeck J, Zazzi M, Camacho RJ, Torti C, et al. HIV-1 Subtype Is an Independent Predictor of Reverse Transcriptase Mutation K65R in HIV-1 Patients Treated with Combination Antiretroviral Therapy Including Tenofovir. Antimicrob Agents Chemother. 2013 Feb; 57(2):1053–1056. [PubMed: 23183438]
11. Sunpath H, Wu B, Gordon M, Hampton J, Johnson B, Moosa MY, et al. High rate of K65R for antiretroviral therapy-naive patients with subtype C HIV infection failing a tenofovir-containing first-line regimen. AIDS. 2012 Aug 24; 26(13):1679–1684. [PubMed: 22739389]
12. Hamers RL, Sigaloff KC, Wensing AM, Wallis CL, Kityo C, Siwale M, et al. Patterns of HIV-1 drug resistance after first-line antiretroviral therapy (ART) failure in 6 sub-Saharan African countries: implications for second-line ART strategies. Clin Infect Dis. 2012 Jun; 54(11):1660–1669. [PubMed: 22474222]
13. Charalambous S, Grant AD, Day JH, Pemba L, Chaisson RE, Kruger P, et al. Establishing a workplace antiretroviral therapy programme in South Africa. AIDS Care. 2007 Jan; 19(1):34–41. [PubMed: 17129856]
14. Innes C, Hamilton R, Hoffmann CJ, Hippner P, Fielding K, Grant AD, et al. A novel HIV treatment model using private practitioners in South Africa. Sex Transm Infect. 2012 Mar; 88(2): 136–140. [PubMed: 22345028]
15. Pillay V, Ledwaba J, Hunt G, Rakgotho M, Singh B, Makubalo L, et al. Antiretroviral drug resistance surveillance among drug-naive HIV-1-infected individuals in Gauteng Province, South Africa in 2002 and 2004. Antivir Ther. 2008; 13(Suppl 2):101–107. [PubMed: 18575198]
16. Zhou Z, Wagar N, DeVos JR, Rottinghaus E, Diallo K, Nguyen DB, 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(11):e28184. [PubMed: 22132237]
17. Johnson VA, Calvez V, Gunthard HF, Paredes R, Pillay D, Shafer R, et al. 2011 update of the drug resistance mutations in HIV-1. Top Antivir Med. 2011 Nov; 19(4):156–164. [PubMed: 22156218]

18. Gallant JE, DeJesus E, Arribas JR, Pozniak AL, Gazzard B, Campo RE, et al. Tenofovir DF, emtricitabine, and efavirenz vs. zidovudine, lamivudine, and efavirenz for HIV. N Engl J Med. 2006 Jan 19; 354(3):251–260. [PubMed: 16421366]

19. Sax PE, Tierney C, Collier AC, Fischl MA, Mollan K, Peeples L, et al. Abacavir-lamivudine versus tenofovir-emtricitabine for initial HIV-1 therapy. N Engl J Med. 2009 Dec 3; 361(23): 2230–2240. [PubMed: 19952143]

20. Margot NA, Lu B, Cheng A, Miller MD. Resistance development over 144 weeks in treatment-naive patients receiving tenofovir disoproxil fumarate or stavudine with lamivudine and efavirenz in Study 903. HIV Med. 2006 Oct; 7(7):442–450. [PubMed: 16925730]

21. DART Virology Group and Trial Team. Virological response to a triple nucleoside/nucleotide analogue regimen over 48 weeks in HIV-1-infected adults in Africa. AIDS. 2006 Jun 26; 20(10): 1391–1399. [PubMed: 16791013]

22. Gallant JE, Staszewski S, Pozniak AL, DeJesus E, Suleiman JM, Miller MD, et al. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. JAMA. 2004 Jul 14; 292(2):191–201. [PubMed: 15249568]

23. Margot NA, Isaacson E, McGowan I, Cheng AK, Schooley RT, Miller MD. Genotypic and phenotypic analyses of HIV-1 in antiretroviral-experienced patients treated with tenofovir DF. AIDS. 2002 Jun 14; 16(9):1227–1235. [PubMed: 12045487]

24. Pozniak A. Tenofovir: what have over 1 million years of patient experience taught us? Int J Clin Pract. 2008 Aug; 62(8):1285–1293. [PubMed: 18705824]

25. Kagan RM, Lee TS, Ross L, Lloyd RM Jr, Lewinski MA, Potts SJ. Molecular basis of antagonism between K70E and K65R tenofovir-associated mutations in HIV-1 reverse transcriptase. Antiviral Res. 2007 Sep; 75(3):210–218. [PubMed: 17442410]

26. Delaugerre C, Flandre P, Marcelin AG, Descamps D, Tamalet C, Cottalorda J, et al. National survey of the prevalence and conditions of selection of HIV-1 reverse transcriptase K70E mutation. J Med Virol. 2008 May; 80(5):762–765. [PubMed: 18360911]

27. Van Rompay KK, Johnson JA, Blackwood EJ, Singh RP, Lipscomb J, Matthews TB, et al. Sequential emergence and clinical implications of viral mutants with K70E and K65R mutation in reverse transcriptase during prolonged tenofovir monotherapy in rhesus macaques with chronic RT-SHIV infection. Retrovirology. 2007; 4:25. [PubMed: 17417971]

Antivir Ther. Author manuscript; available in PMC 2014 June 10.

Table 1

Characteristics by initial ART regimen

	Whole cohort n (%) or median (IQR)	Initial NRTI prior to switch to TDF n (%) or median (IQR)			p*
		AZT	d4T	TDF	
N	1682	206	836	640	
Men	843 (50)	131 (59)	202 (24)	510 (80)	0.0001
Age (years), median	37 (32, 45)	40 (33, 47)	36 (31, 42)	39 (33, 47)	0.0001
CD4 count at ART initiation, cells/mm3	194 (108, 328)	206 (128, 347)	160 (80, 256)	261 (152, 394)	0.0001
HIV RNA at ART initiation, $\log_{10}$ c/mL	4.7 (4.5, 4.9)	4.7 (4.4, 4.8)	4.7 (4.6, 4.8)	4.7 (4.5, 5.0)	0.2
Median time on initial NRTI prior to TDF, days	639 (352, 1,005)	791 (445, 1,187)-		0.005	
Virologic failures, n	270	56	66	148	
Time on TDF at failure, median, days	152 (97, 222)	152 (106, 222)	160 (93, 219)	141 (91, 224)	0.6
CD4 count at TDF failure, median, cells/mm^3	199 (121, 303)	174 (124, 239)	215 (125, 356)	205 (120, 308)	0.1
HIV RNA at failure on TDF, median, $\log_{10}$ c/mL	4.3 (3.6, 4.9)	4.4 (4.1, 4.8)	4.1 (3.4, 4.8)	4.3 (3.6, 5.0)	0.2
Number of failures with genotyping	40	8	19	13	
K65R	5	0	5 (26)	0	0.06
M184V	11	2 (25)	7 (37)	2 (15)	0.4
NNRTI	22	6 (62)	11 (58)	5 (38)	0.5
AS-PCR K65R	9	1 (12)	6 (32)	2 (15)	0.5
AS-PCR M184V	10	2 (25)	5 (28)	3 (23)	1.0

NRTI: nucleoside reverse transcriptase inhibitor; AZT: zidovudine; d4T: stavudine; TDF: tenofovir disoproxil fumarate; AS-PCR: allele-specific polymerase chain reaction.

*p for difference between initial NRTI. Comparison of continuous variables using the Kruskal-Wallis method and proportions using the chi-square test.

Table 2

Resistance Mutations

	Population Sequencing Number (%; 95% Confidence Interval)	Mutations *only* identified by Allele-specific PCR
N	**40**	
Major NNRTI mutation	**21 (52; 36–68)**	
K101E	1	
K103N	15	
V106M	4	
V108I	3	
V179D	1	
Y181C	3	
G190A	3	
P225H	3	
F227L	1	
M230L	1	
M184V/I	**11 (28; 15–44)**	**1**
K65R	**5 (12; 4.1–27)**	**5**
Any TAM	**4 (10; 2.8–24)**	
M41L	1	
D67N	2	
K70R	1	
L210W	1	
T215Y	1	
K219Q	1	

Table 3

Associations with resistance (identified by sequencing) by mutation

	K65R n or median (IQR)			M184V n or median (IQR)			Major NNRTI resistance n or median (IQR)		
Mutation present (n)	**No (38)**	**Yes (5)**	**P***	**No (31)**	**Yes (12)**	**P***	**No (19)**	**Yes (24)**	**P***
Prior ART regimen (n)									
No	12	0	0.3	11	1	0.08	8	4	0.1
Yes	23	5		18	10		11	17	
Prior d4T (n)									
No	**21**	**0**	**0.02**	17	4	0.3	11	10	0.5
Yes	**14**	**5**		12	7		8	11	
Total ART duration, days: median (IQR)	935 (624, 1291)	744 (515, 1100)	0.5	840 (536, 1115)	1136 (744, 1515)	0.3	825 (492, 1115)	1064 (700, 1290)	0.2
TDF duration, days, median (IQR)	224 (163, 402)	178 (112, 219)	0.3	235 (151, 402)	198 (168, 262)	0.7	235 (168, 438)	198 (122, 324)	0.5
CD4 at failure, cells/mm^3, median (IQR)	218 (102, 331)	337 (233, 366)	0.2	159 (58, 350)	163 (79, 218)	0.4	250 (112, 327)	218 (107, 348)	0.9
HIV RNA at genotyping, log_{10} c/mL, median (IQR)	**4.2 (3.8, 5.0)**	**3.3 (3.2, 3.7)**	**0.02**	**4.3 (3.8, 5.1)**	**3.4 (3.1, 3.9)**	**0.004**	**4.5 (3.8, 5.2)**	**3.7 (3.2, 4.5)**	**0.02**