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MAJOR ARTICLE

Persistence of Human Immunodeficiency Virus-1 Drug Resistance Mutations in Proviral Deoxyribonucleic Acid After Virologic Failure of Efavirenz-Containing Antiretroviral Regimens

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Background. Efavirenz (EFV)-based regimens select broad drug resistance to nonnucleoside reverse-transcriptase inhibitors (NNRTIs), limiting the effectiveness of EFV and other NNRTIs. The duration, persistence, and decay of drug resistance mutations (DRMs) in the proviral reservoir is not well defined.

Methods. Participants with virologic failure of EFV-based regimens and drug-resistant viremia with the K103N mutation in plasma ribonucleic acid (RNA) were identified from AIDS Clinical Trials Group (ACTG) studies A364 and A5095. These individuals received a second-line, boosted protease inhibitor-based regimen with suppression of viremia for up to10 years during long-term follow-up (median = 3.6 years; interquartile range, 2.1–6.9 years). Proviral deoxyribonucleic acid (DNA) from cryopreserved peripheral blood mononuclear cells was sequenced to identify the persistence of DRM.

Results. Twenty-eight participants from ACTG 364 and ACTG 5095 were evaluated. Sanger sequencing of proviral DNA detected K103N as well as additional reverse-transcriptase inhibitor (RTI) mutations. Ultradeep sequencing confirmed persistence of K103N in 71% of participants with minimal decay over time. In an adjusted model including years since suppression, persistent proviral K103N was 2.6 times more likely (95% confidence interval, 1.0–6.4) per $\log_{10}$ higher human immunodeficiency virus RNA at EFV failure.

Conclusions. Persistence of RTI mutations in proviral DNA after virologic failure has implications for the effectiveness of future drug regimens and the recycling of RTI drugs.

Keywords. drug resistance mutations; efavirenz; nonnucleoside reverse-transcriptase inhibitors; proviral DNA.

With the rapid scale-up of antiretroviral therapy (ART), more than 20 million people will receive a first-line, efavirenz (EFV)-based regimen by 2020 [1]. Until recently, in low- and middle-income countries (LMIC) the recommended first-line treatment was EFV, tenofovir, and lamivudine [2]. Studies of drug resistance mutations (DRMs) in plasma virus ribonucleic acid (RNA) by Sanger consensus sequencing frequently identify mutations associated with nonnucleoside reverse-transcriptase inhibitors (NNRTIs); most often K103N, followed by G190S, V106A/M, Y181C, Y188L, and P225H [3, 4]. These observations have largely depended on commercial sequencing assays (ViroSeq Genotyping System [Applied Biosystems, Foster City, CA], TRUGENE HIV-1 Genotyping Kit [Visible Genetics, Inc., Toronto, Ontario, Canada]) and laboratory-developed assays of viral RNA resistance that are typically limited to plasma samples with RNA virus loads >1000–2000 copies/mL [5–8].

After first-line, virologic failure, a boosted protease inhibitor (PI) and 2 nucleoside reverse-transcriptase inhibitors (NRTIs) are recommended to achieve and maintain suppression of human immunodeficiency virus (HIV) RNA [9]. However, despite suppression of HIV RNA to <50 copies/mL on effective ART, HIV proviral deoxyribonucleic acid (DNA) in circulating CD4 cells decays slowly over the first years of treatment [10]. Sequencing proviral DNA has demonstrated archival resistance mutations from past treatments [11, 12] in cellular reservoirs [13] with phylogenetic transmission clustering [14], viral evolution [15], and may determine eligibility for drug switching among suppressed patients [16–18]. The sensitivity of HIV

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DNA genotyping is limited by the frequency of latent infection in memory CD4 lymphocytes. Recent studies of the CD4 reservoir of suppressed patients have consistently found 100–1000 (2.0–3.0 $\log_{10}$ copies) of HIV-1 DNA per microgram of peripheral blood mononuclear cell (PBMC) DNA from infants [19] and adults [10, 20], even after years of suppression. Cloning proviral DNA demonstrates that only 10%–12% of provirus is complete and potentially replication competent [21], and viral outgrowth assays from suppressed patients may demonstrate an additional 5- to 10-fold reduction in replication-competent virus compared with semiquantitative polymerase chain reaction (PCR) assays. However, DRMs in archival DNA have the potential to recombine with the emergence of resistance, regardless of the viability of the archival quasispecies [22].

To evaluate the retention and decay of DRMs after the emergence of plasma resistance, we identified participants with known dates of failure of an EFV-based regimen with the K103N mutation in plasma RNA by population sequencing. These participants were (1) enrolled in AIDS Clinical Trials Group (ACTG) clinical trials, (2) subsequently treated with an effective, suppressive second-line, boosted PI regimen (lopinavir/ritonavir [LPV/r]), and (3) followed for up to 10 years (median = 3.6 years; interquartile range [IQR], 2.1–6.9 years). Peripheral blood mononuclear cell samples after suppression were analyzed to follow the emergence, fixation, retention, and decay of DRM in proviral DNA using Sanger and next-generation sequencing. Drug-resistant viruses persist as minority quasispecies that may emerge with treatment interruption or virologic failure with implications for design of salvage therapies and transmission of drug resistance.

MATERIALS AND METHODS

Study Population

Twenty-nine participants were selected on an EFV-based regimen, with a K103N mutation in plasma RNA by population sequencing from ACTG 364 [23] and 5095 [24]. All had consented to continued participation in the ACTG Longitudinal Linked Randomized Trials (ALLRT) protocol, and 28 were successfully suppressed on LPV/r regimens after viral failure with K103N [25]. One patient withdrew because of virologic failure on LPV/r (participant 26). CD4 count and viral load were measured, and PBMCs were collected and cryopreserved every 6 months.

Proviral Deoxyribonucleic Acid Amplification and Sequencing

For most participants' samples, genotypes were obtained at 3 time points after LPV/r suppression—early suppression (t_1), midpoint (t_2), and latest suppression (t_3)—and the consensus RT sequence at each time point was compared with the initial RNA sequence at virologic failure. The median intervals from suppression to t_1, t_1 to t_2, and t_2 to t_3 for participants with all time points were 0.6 years (IQR, 0.6–0.7), 1.9 years (IQR, 1.0–3.3), and 1.9 years (IQR, 0.9–3.1), respectively.

For PBMC genotyping, DNA was extracted from pelleted cells using the QIAamp DNA Blood Mini Kit (QIAGEN) according to the manufacturer's recommendations. In brief, 20 µL Protease K and 200 µL 100% ethanol were added, and the lysate was incubated at 56°C for 10 minutes before elution from the purification column.

The *pol* gene from proviral DNA was amplified using 2 rounds of PCR with Platinum Taq polymerase (Invitrogen). Primers MAW26 (5'-TTGGAAATGTGGA AAGGAAGGAC-3') and RT21 (5'-CTGTATTTCTG CTATTAAGTCTTTTGATGGG-3') were used at 500 nM concentration. The following conditions were used for the first round of PCR: 95°C for 2 minutes, 30 cycles of 94°C for 15 seconds, 55°C for 20 seconds, and 72°C for 2 minutes, concluding with 1 cycle of 72°C for 10 minutes. Five microliters of purified PCR product from the first round of PCR were used as the template for the second round of PCR using the same thermocycler parameters. Primers PRO1 (5'-CAGAGCCAA CAGCCCCACCA-3') and RT20 (5'-CTGCCAGTTCT AGCTCTGCTTC-3') were used at 500 nM concentration. The purified PCR product was then sequenced by Sanger methods (MCLAB, South San Francisco, CA) using primers MAW70 (5'-TAATCCCTGCGTAAATCTGACTTGCCCA-3'), RTA (5'-GTTGACTCAGATTGGTTGCAC-3'), RTB (5'-CCTAGTATAAACAATGAGACAC-3'), and RTY (5'-GGATCATATTT GTTACTCTGTG-3') and analyzed using Geneious 6.1.7 software (Biomatters, New Zealand).

Limiting Dilution Polymerase Chain Reaction Quantification of Provirus

Peripheral blood mononuclear cells were lysed to a concentration of 10^5 cells/µL, and serial 5-fold dilutions were amplified in triplicate to estimate the copies of HIV DNA per 10^6 PBMCs [26], as previously described [27].

Phylogenetic Analysis

Assembled sequences were aligned and edited using Geneious 6.1.7. Neighbor-joining trees containing all *pol* sequences were constructed using the F84 model of nucleotide substitution using PHYLIP 3.69 software (University of Washington, Seattle, WA). The trees were rooted to consensus B sequence and 100 bootstrap replicates were performed.

Next-Generation Sequencing

Regions of reverse transcriptase were amplified as size-appropriate amplicons to barcode for multiplex paired-end Illumina (San Diego, CA) MiSeq sequencing. Primers MAW26 (TTGGAAATGTGGAAAGGAAGGAC) and RT21 (CTGTATTT CTGCTATTAAGT CTTTTGATGGG) were used for the first round of replication. The following conditions were used for the first round of PCR: 95°C for 2 minutes, 35 cycles of

94°C for 15 seconds, 55°C for 20 seconds, and 72°C for 2 minutes, concluding with 1 cycle of 72°C for 10 minutes. Primers with adapters and indices were used for the second round of replication. The forward primers include the flow-cell attachment sequence (italics) and a custom Read1 sequencing primer (underlined) as prefix: 5'-*AATGATACGGCGACCACCGA* GATCTACACCC TACACGAGCG TTATCGAGGTC-3'.

The reverse primers include the flow-cell attachment sequence (italics), index, and the default Read 2 sequencing primer (underlined) as prefix: 5'-*CAAGCAGAAGAC GGCATACGAGAT* - (index) – GTGACTGGA GTTCAGA CGTGTGCTCTTCCGATCT-3'. The following primers amplify reverse transcriptase into 3 amplicons: 41–74 Forward CCRAAAAGTTA AACAATGGCC; 41–74 Reverse GGTATTCCTAATTGBACTTCC; 100–151 Forward GGAAGTVCAATTAGGAATACC; 100–151 Reverse GCTACVTTGGAATATTGCTGG; 181–230 Forward GCATGACAAAAATCTTAGADCC; 181–230 Reverse CNYTATAGGCTGTACTGTCC. The following conditions were used for the second round of PCR: 95°C for 2 minutes, 5 cycles of 94°C for 15 seconds, 45°C for 20 seconds, and 72°C for 15 seconds followed by 30 cycles of 94°C for 15 seconds, 60°C for 20 seconds, and 72°C for 15 seconds, concluding with 1 cycle of 72°C for 1 minute.

Amplicon libraries were purified using the GeneJET PCR Purification Kit (Thermo Fisher Scientific Inc., Waltham, MA) and run on the 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) to assess the size and quality of DNA. Before library pooling, barcoded amplicons were quantified using the Qubit dsDNA HS assay (Thermo Fisher Scientific). The library of pooled amplicons was prepared at a final concentration of 4 nM, denatured with 0.2 N NaOH for 5 minutes, and diluted to a final concentration of 10 pM. The denatured library was spiked with 40% denatured PhiX control library (Illumina) and subjected to 250 cycles of paired-end sequencing using the MiSeq reagent kit, version 2.0 (Illumina) per the manufacturer's protocol. A custom Read1 sequencing primer was used.

Table 1. Characteristics of the Participants Enrolled in ACTG Studies Who Failed EFV[a]

ID	Study	Virus Load at K103N, Log_{10} RNA Copies/mL plasma	CD4 at K103N, Cells/mm^3	Years Suppressed on ART	Sanger K103N DNA at Latest Timepoint	Illumina K103N DNA at Latest Timepoint
1	A364	3.72	211	10.93	Not Detected	Not Detected
2	A364	2.60	492	6.96	Detected	Detected
3	A364	4.72	273	5.18	Detected	Detected
4	A364	3.69	385	4.56	Not Detected	Not Detected
5	A364	4.70	561	2.14	Not Detected	Not Detected
6	A364	4.28	161	1.86	Not Detected	Not Detected
7	A364	3.43	588	1.2	Not Detected	Not Detected
8	A364	3.31	628	0.78	Not Detected	Not Detected
9	A5095	3.37	N/A	10.26	Detected	Detected
10	A5095	5.06	N/A	9.55	Not Detected	Detected
11	A5095	3.85	109	8.78	Not Detected	Not Detected
12	A5095	3.54	543	7.92	Not Detected	Not Detected
13	A5095	4.18	N/A	7.13	Detected	Detected
14	A5095	3.27	N/A	6.86	Not Detected	Not Detected
15	A5095	3.35	N/A	4.92	Not Detected	Not Detected
16	A5095	3.95	225	4.77	Detected	Detected
17	A5095	3.62	N/A	4.67	Not Detected	Not Detected
18	A5095	4.22	N/A	3.85	Not Detected	Detected
19	A5095	5.62	612	3.42	Detected	Detected
20	A5095	4.53	401	3.38	Detected	Detected
21	A5095	4.61	221	2.91	Detected	Detected
22	A5095	5.14	259	2.76	Detected	Detected
23	A5095	3.88	N/A	2.51	Not Detected	Detected
24	A5095	2.73	459	2.46	Detected	Detected
25	A5095	4.64	432	1.86	Detected	Detected
26	A5095	Withdrawn	Withdrawn	N/A	N/A	N/A
27	A5095	3.12	N/A	1.01	Not Detected	Not Detected
28	A5095	5.99	N/A	0.92	Detected	Detected
29	A5095	4.48	767	0.35	Detected	Detected

Abbreviations: ACTG, AIDS Clinical Trials Group; ART, antiretroviral therapy; DNA, deoxyribonucleic acid; IQR, interquartile range; N/A, not applicable; RNA, ribonucleic acid.

[a]The first 8 participants from ACTG 364 had a long history of dual nucleoside treatment, and those from ACTG 5095 were naive. All participants received a second-line, boosted protease inhibitor-based regimen with suppression of viremia for up to 10 years during long-term follow-up (median, 3.6 years; IQR, 2.1–6.9 years). The last 2 columns indicate whether K103N was detected at the latest time point by Sanger and Illumina sequencing, respectively.

FASTQ files were aligned to the HIV-1 (HXB2) reference sequence (K03455.1) using Burroughs-Wheeler Aligner (BWA) software (version 0.7.12) with the BWA-Smith Waterman algorithm and default parameters [28]. A custom python script using pysam, version 0.12 (available upon request), scanned the BAM alignment files to generate codon-wise pileup counts. The codons that differed from the reference sequence were considered variants and were translated into amino acids. Drug resistance mutations were expressed as the number of mutant codons observed divided by the total read depth at that codon position multiplied by 100. The noise threshold was empirically set at a conservative 2%, and variants observed below the 2% level were considered error.

Statistical Analysis

Repeated measures logistic regression (generalized estimating equations) was used to identify factors associated with detectable K103N, or other DRMs, in proviral DNA [29]. Nucleoside reverse-transcriptase inhibitor DRMs included M41I/L, D67N/E, K70R, M184V, T215Y/F/C/S, K219Q/E; NNRTI DRM included K103N, Y181C, G190A/S/R, L100I, K101E, V106I/M, Y188H/C/L, M230I/L [7].

RESULTS

Participant Characteristics

Serial samples of PBMCs were evaluated from consenting participants enrolled in ACTG studies between 1996 and 2005 and subsequently followed in the ALLRT study. The first 8 participants had been extensively treated with dual nucleosides in ACTG studies 175, 302, 303, and 364 before commencing an EFV-based 3-drug regimen in ACTG 364 [30]. In contrast, the 26 participants enrolled in ACTG 5095 were drug naive before EFV, azidothymidine (AZT), and lamivudine (3TC) treatment [24]. Sequencing at virologic failure included the K103N mutation among 28 participants. The $\log_{10}$ virus load and CD4 count at virologic failure are shown in Table 1, as are the duration of suppression on LPV/r and 2 nucleosides and the evaluation of provirus K103N by Sanger and Illumina sequencing at the last available timepoint.

Virologic Failure on an Efavirenz-Based Regimen

Sanger sequencing was performed on plasma samples (in retrospect) at virologic failure, defined as the second of 2 virus load measurements >200 copies/mL, at least 2 weeks apart. The EFV-based regimen assigned in the clinical study was continued for 0.5 to more than 10 years for a median of 9.5 months (IQR, 4.0–20.5). The virus load at the time of confirmed virologic failure (at the identification of the K103N mutation) varied from 2.73 to 5.99 $\log_{10}$ copies/mL ethylenediaminetetraacetic acid plasma (geometric mean, 4.04 $\log_{10}$ copies/mL; median, 3.98 $\log_{10}$ copies/mL; IQR, 3.43–4.55 $\log_{10}$ copies/mL). Suppressive LPV/r-based (PI) therapy was initiated with prompt reduction in viremia to <50 copies/mL. Continued 6-monthly follow-up on the ALLRT study provides evidence that suppression was maintained in all 28 participants.

Nonnucleoside Reverse-Transcriptase Inhibitor and Nucleoside Reverse-Transcriptase Inhibitor Drug-Resistant Mutations in Plasma RNA at Virologic Failure and Proviral DNA After Suppression via Sanger Sequencing

Phylogenetic distance between plasma RNA sequence at failure and proviral DNA after suppression were analyzed (Supplementary Figure S1). As expected, the plasma RNA and proviral DNA sequences demonstrated <1% dissimilarity within each individual. Differences between the plasma RNA and DNA identified by branch length in the tree were not different comparing the mean DNA-RNA genetic distance, between the 12 sequences with K103N in proviral DNA and the 14 sequences without K103N.

Sequences of plasma RNA at failure demonstrated high-level NNRTI DRM in all 28 participants with K103N; NRTI DRMs were identified in plasma RNA in 75% of these participants (Figure 1). Frequency of participants with NNRTI and NRTI mutations as detected by Sanger sequencing by time point demonstrated only a modest decrease in the percentage of participants with detectable K103N over time (Figure 1). Detection of NRTI mutations in DNA decreased with time on suppression ($P < .01$). Years since start of suppression was not associated with the likelihood of detectable K103N ($P = .53$). Nonnucleoside reverse-transcriptase inhibitor DRMs persisted in the proviral DNA at similar levels over time (Figure 2A). It is interesting to

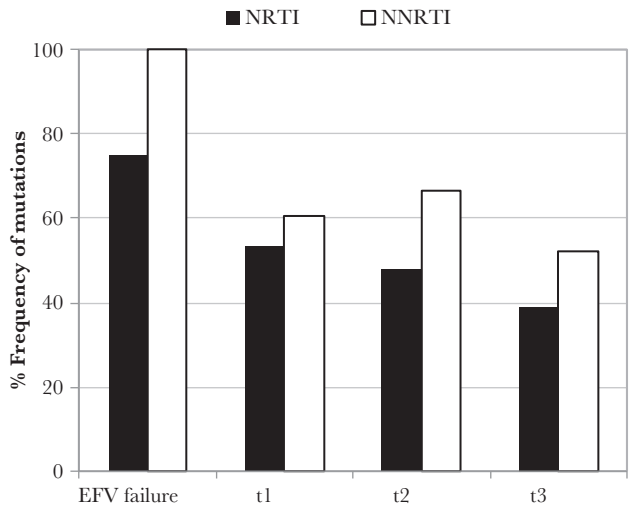

Figure 1. Frequency of participants with nonnucleoside reverse-transcriptase inhibitor (NNRTI) and nucleoside reverse-transcriptase inhibitor (NRTI) mutations by time point as detected by Sanger sequencing. Percentage of participants with detectable NNRTI versus NRTI mutations at efavirenz (EFV) failure, early suppression (t_1), midpoint suppression (t_2), and latest suppression (t_3). Mutations at EFV failure were detected in ribonucleic acid, whereas mutations identified after suppression were detected in proviral deoxyribonucleic acid.

note that M184V was retained in proviral DNA, whereas other NRTI DRM demonstrated modest decreases (Figure 2B).

In an adjusted model including years since suppression, $\log_{10}$ plasma HIV RNA level at EFV failure was associated with increased likelihood of observing proviral K103N ($P = .04$); odds of detectable proviral K103N was 2.6 times higher (95% confidence interval, 1.0–6.4) per $\log_{10}$ higher HIV RNA at EFV failure.

Detection of Low-Level Drug-Resistant Mutations by Illumina Sequencing After Suppression

Deep sequencing of proviral DNA demonstrated that 50% (14 of 28) of participants retained K103N at levels >20%. Another 21% (6 of 28) of participants had K103N minority variants at levels between 2% and 20% (total retention 71% [20 of 28]). Finally, K103N was not detected in the proviral DNA of 29% (8 of 28) participants who had K103N in plasma RNA at virologic failure. Sanger sequencing showed that K103N was retained in a total of 61% (17 of 28) of participants. Likewise, M184V was detected by deep sequencing at levels >20% in 25% (7 of 28) of participants, 14% (4 of 28) at levels between 2% and 20% [total retention 39% (11 of 28)], and was not detected in 61% (17 of 28) of participants. Sanger sequencing revealed that M184V was retained in 25% (7 of 28) of participants.

We also used deep sequencing to analyze K103N and M184V levels within individuals to assess persistence and decay over time. In most cases, levels remained consistent within the categories of not detected, minority DRM (2%–20%), and >20%. The exceptions were participants with decay from high level to <20% (K103N, n = 2; M184V, n = 1), participants whose levels went from undetectable to >20% (K103N, n = 1; M184V, n = 1), and participants whose levels went from undetectable to >20% back to undetectable (K103N, n = 0; M184V, n = 1) (Figure 3). Full data for all analyzed mutations can be found in Supplemental Figures 2 and 3.

DISCUSSION

Individuals infected with HIV increasingly may carry a long treatment history of exposure to first-line ART, particularly EFV and 2 NRTIs, the recommended "cocktail" for approximately 20 years. Although ART can reduce and sustain plasma HIV-1 RNA to undetectable levels, viral replication and viremia

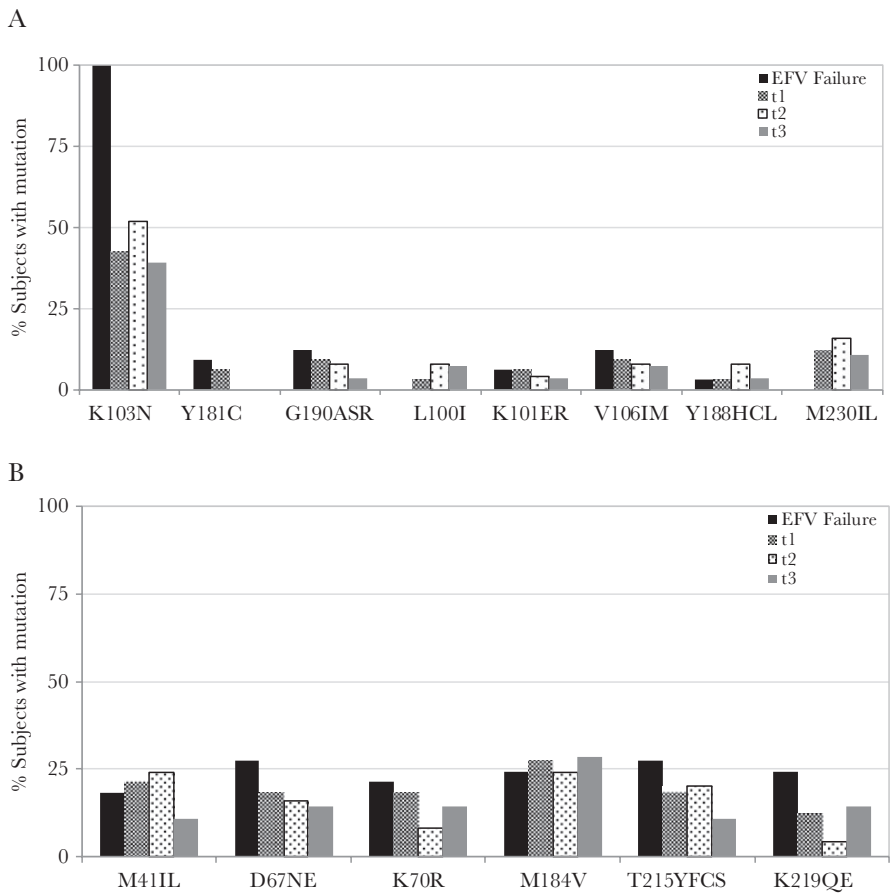

Figure 2. Retention of drug resistance mutations in proviral deoxyribonucleic acid during suppression as detected by Sanger sequencing. Percentage of participants with detectable (A) nonnucleoside reverse-transcriptase inhibitor and (B) nucleoside reverse-transcriptase inhibitor mutations at efavirenz (EFV) failure, early suppression (t_1), midpoint suppression (t_2), and latest suppression (t_3) detected through Sanger population sequencing.

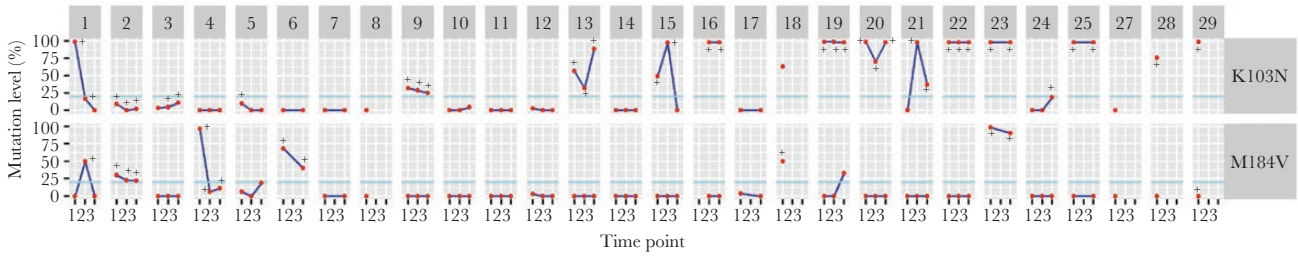

Figure 3. K103N and M184V drug resistance mutations by time point as detected by Illumina sequencing. Level of K103N and M184V drug resistance mutations for each participant at early suppression (1), midpoint suppression (2), and latest suppression (3) detected through Illumina sequencing at a threshold of 2%. The 20% level is shown as a light blue line. All 3 time points were not available for all participants. Those with limited follow up had only a single time point. Time points at which K103N or M184V were also detected by Sanger sequencing are indicated by plus signs (+).

resumes if treatment is stopped, drug levels become inadequate, or a drug-resistant virus is introduced through sexual or parenteral exposure. Return of replication and viremia is due to the reactivation of T cells harboring latent, integrated provirus, the HIV-1 reservoir. This latent reservoir, largely, if not wholly in Th-1 CD4 cells, is the primary barrier to "cure" or eradication of HIV. Moreover, as antiretroviral regimen switching is considered for HIV-1-infected, virologically suppressed patients, to reduce long-term toxicities or improve tolerability, ART for long-term maintenance of viral suppression may be guided by identification of archival drug resistance.

Recent studies of individuals with virologic suppression have demonstrated HIV genomic DNA in T cells, including naive CD4 cells, central memory, and effector memory cells that contain replication-competent provirus for many years [31]. In this study, we focused on the detection of the K103N mutation in proviral DNA, the drug-resistant mutation most frequently associated with virologic failure of EFV-based regimens. Among participants who had failed EFV+AZT+3TC with K103N in plasma RNA, 50% had detectable K103N in archival proviral DNA by Sanger sequencing for as long as 8 years after viral suppression with a PI-based regimen. Next-generation sequencing has been increasingly used to assess minority variant DRMs at low levels (>2 to <20%) associated with transmitted or acquired resistance mutations in plasma RNA and predictive of virologic failure [32, 33]. In this study, deep sequencing, albeit of a limited number of proviruses, demonstrated an additional 21% of participants with minority levels of K103N.

The positive association between plasma HIV RNA level at viral failure and detection of K103N in proviral DNA is consistent with the maintenance of proviral DNA in PBMCs [10], the association between pre-ART plasma HIV RNA level and the on-ART level of proviral HIV DNA [34], and the well characterized persistence of K103N after discontinuation of NNRTI selection pressure [35, 36].

Detection of resistance mutations in archival proviral DNA after prolonged suppression may be limited by the relatively small number of circulating T cells with provirus sampled and does not exclude resistant proviruses in lymphoid tissue or other tissue reservoirs. In general, reuse or recycling of first-generation NNRTIs after a history of failure of this class of drug is not recommended. However, exposure to single-dose nevirapine (NVP) or unknown ART treatment(s) or transmitted resistance may result in archived DRMs that impair responses to EFV [35, 37, 38]. However, second-generation NNRTIs, rilpivirine (RPV) and etravirine (ETR), may still be considered in salvage regimens in the absence of evidence for resistance [39]. With phenotypic testing, even isolates with mutations associated with EFV and NVP resistance demonstrated 51% and 43% phenotypic susceptibility to second-generation NNRTIs ETR and RPV, respectively [40]. The recent approval of doravirine, with activity against many NNRTI-resistant viruses, may provide an additional treatment option after EFV failure [41].

CONCLUSIONS

Deep sequencing of proviral genomes from PBMCs provides evidence that evaluation of proviral DNA may be a useful measure of the drug resistance burden, particularly after virologic failure [42]. The proviral reservoir may be stable with a half-life of up to 44 months [43, 44]. In contrast, Sanger sequencing of plasma RNA in the absence of drug pressure may demonstrate a decline or persistence of transmitted drug mutations depending on the drug class and the fitness of the mutation [45, 46]. More recent studies of the stability of HIV-latent reservoirs in $CD4^+$ T cells [21, 47, 48] and the decay rates of DRM in the latent reservoir suggest that genotyping of suppressed patients with "undetectable" plasma RNA is feasible and proviral genotype could guide drug switching and the implementation of new drug regimens.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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