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Minority Variants Associated with Transmitted and Acquired HIV-1 Non-Nucleoside RT Inhibitor (NNRTI) Resistance: Implications for the Use of Second Generation NNRTIs

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Abstract

Objectives—K103N, the most common nonnucleoside (NNRTI)-resistance mutation in patients with transmitted resistance and in patients receiving a failing NNRTI-containing regimen, is fully susceptible to the new NNRTI etravirine. Therefore, we sought to determine how often NNRTI-resistance mutations other than K103N occur as minority variants in plasma samples for which standard genotypic resistance testing (SGRT) detects K103N alone.

Methods—We performed ultradeep pyrosequencing (UDPS; 454 Life Sciences a Roche Company) of plasma virus samples from 13 treatment-naïve and 20 NNRTI-experienced patients in whom SGRT revealed K103N but no other major NNRTI-resistance mutations.

Results—Samples from 0 of 13 treatment-naïve patients vs 7 of 20 patients failing an NNRTI-containing regimen had minority variants with major etravirine-associated NNRTI resistance mutations (p=0.03, Fishers Exact Test): Y181C (7.0%), Y181C (3.6%) + G190A (3.2%), L100I (14%), L100I (32%) + 190A (5.4%), K101E (3.8%) + G190A (4.9%), K101E (4.0%) + G190S (4.8%), and G190S (3.1%).

Conclusion—In treatment-naïve patients, UDPS did not detect additional major NNRTI-resistance mutations suggesting that etravirine may be effective in patients with transmitted K103N. In NNRTI-experienced patients, UDPS often detected additional major NNRTI-resistance mutations suggesting that etravirine may not be fully active in patients with acquired K103N.

Keywords

Reverse transcriptase (RT); Nonnucleoside RT inhibitors; Drug resistance mutations; Etravirine; Pyrosequencing; Quasispecies

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Introduction

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are essential components of antiretroviral (ARV) therapy [1]. However, NNRTIs have a low genetic barrier to resistance: a single mutation is often sufficient to cause resistance to the currently recommended first generation NNRTIs, nevirapine and efavirenz. The RT mutation K103N is the most commonly occurring NNRTI-resistance mutation in patients with acquired 2 3 and transmitted 4 [5] NNRTI resistance. K103N reduces susceptibility to efavirenz and nevirapine by ~20-fold and ~50-fold, respectively[3], but has no effect on susceptibility to the most recently approved NNRTI, etravirine[6].

As a result of the low genetic barrier to NNRTI resistance, multiple NNRTI-resistant lineages often emerge in plasma samples from patients experiencing ongoing viral replication while receiving an NNRTI-containing regimen7[-9]. Standard genotypic resistance testing (SGRT) performed by direct PCR sequencing typically detects HIV-1 variants comprising ≥20% of the viruses within a clinical sample but may miss less-prevalent drug resistant variants. To determine whether minority etravirine-resistance variants are present in patient samples, we performed ultra-deep pyrosequencing (UDPS; 454 Life Sciences, a Roche Company, FLX technology) of plasma samples from treatment-naïve and NNRTI-experienced patients for whom direct PCR sequencing detected K103N but no other major NNRTI-resistance mutation.

Methods

Patients and Samples

Patients included HIV-1-infected individuals followed within the Kaiser Permanente Medical Care Program of Northern California (KPNC) who had genotypic resistance tests performed at Stanford University Hospital between August 1998 and December 2007. Patient samples were characterized according to the following criteria: (i) presence of the RT mutation K103N, (ii) ARV treatment history, (iii) plasma HIV-1 RNA level and (iv) availability of a cryopreserved sample.

Two types of samples meeting study criteria were identified: (i) samples from ARV-naive patients containing the K103N mutation ("transmitted K103N") and (ii) samples obtained from patients receiving a failing NNRTI-containing regimen ("acquired K103N"). To be eligible for study, plasma samples had to have plasma HIV-1 RNA levels ≥4.5 log copies/ml and an available cryopreserved sample. Samples with acquired and transmitted K103N were studied only if they lacked other major NNRTI resistance mutations or known etravirine-resistance mutations.

Direct PCR Sequencing

Genotypic resistance testing for clinical purpose was carried out on all samples by direct PCR standard dideoxynucleotide sequencing as previously described [10]. Briefly, RT-PCR products were obtained by plasma virus ultracentrifugation; followed by RNA extraction, reverse transcription using a high-fidelity RT enzyme, and PCR using *Taq* polymerase (The SuperScript One-Step RT-PCR with Platinum *Taq* System, Invitrogen, Carlsbad, CA). Direct PCR bidirectional-sequencing encompassing HIV-1 protease and the first 250 to 350 codons of RT was performed using Big-Dye Terminators with products resolved electrophoretically on an ABI 3100 sequencer (Applied Biosystems, Foster City CA). GenBank accession numbers of 14 previously submitted direct PCR sequences: AY030747, AY031476, AY030906, AF514165, AY030776, AY801580, AY032406, AY801679, AY801946, FJ983276, FJ983294, AY802041, AY802048 and FJ983334. GenBank accession numbers for the remaining 19 of the direct PCR sequences are pending.

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Mutations

Etravirine-resistance mutations were defined as mutations associated in the DUET studies with a decreased virological response to etravirine: V90I, A98G, L100I, K101E/H/P, V106I, E138A, V179D/F/T, Y181C/I/V, G190A/S, and M230L [11, 12]. The mutations V90I, A98G, V106I, E138A, and V179D/T were considered to be less important indicators of etravirine resistance because they are polymorphic (particularly V90I and V106I) and have not been shown to have a phenotypic effect on etravirine susceptibility. L100I, K101E/P, K103N/S, V106A/M, Y181C/I/V, V179F, Y188C/H/L, G190A/S/E/Q and M230L were defined as major NNRTI resistance mutations [13].

Ultra-Deep Pyrosequencing (UDPS)

One ml of plasma was ultracentrifuged for 30 minutes and RNA was extracted using the Roche Amplicor RNA extraction kit according to manufacturer's protocol. Reverse-transcription was performed with random primers and Superscript III RT (Invitrogen, Carlsbad, CA) followed by RnaseH (Invitrogen, Carlsbad, CA) treatment to improve PCR efficiency. A first round PCR was used to amplify a 1337 bp product. Three second round amplifications encompassed protease (positions 8 to 99) and the 5′ part of RT genes (positions 1 to 238). Each primer consisted of a 5′ 19-nucleotide UDPS adaptor, one of seven patient-specific barcodes (ACTT, ATCA, TCTG, TACT, CTCT, CTCA or CTAC), and the 3′ HIV-1 target sequence (Supplementary Table 1). PCR reactions were performed in a 50μl reaction mixture that contained 2.5μl of cDNA, 10μl Expand High FidelityPLUS Reaction Buffer, 2 mmol/L MgCl2, 0.2 mmol/L dNTPs, 0.5 mmol/L of each primer and 2.5 U Expand High Fidelity PLUS DNA polymerase. cDNA titers were estimated by limiting dilution PCR of cDNA to confirm the presence of ≥100 amplifiable virus templates.

PCR products were purified by using AMPure beads (Agencourt Biosciences, Beverly, MA), quantified using Quant-iT Picogreen dsDNA reagent (Invitrogen, Carlsbad, CA), and pooled at equimolar concentrations. Clonal amplification on beads (emulsion PCR) was performed using reagents that enabled sequencing in both the forward and reverse directions (emPCR kits II and III; 454 Life Sciences, Branford, CT). DNA containing beads were isolated and counted on a Multisizer 3 Coulter Counter (Beckman Coulter, Fullerton, CA). UDPS was performed on a Genome Sequencer FLX (454 Life Sciences, Branford, CT), and each sample pool was loaded in 1 region of a 70 mm × 75 mm PicoTiter plate (454 Life Sciences, Branford, CT) fitted with a 4-lane gasket. Four PicoTiter plates were used to sequence 33 clinical samples and 4 plasmid control samples. The samples in the study shared plates with approximately an equal number of samples from other studies.

UDPS Coverage and Technical Error Rate

UDPS generated a median of 14,950 reads per sample with a median read length of 245 bases. This resulted in a median coverage of 3,654 reads per base. An analysis based on five pNL43 clonal sequences performed on the same plates with the clinical samples yielded an overall mismatch error rate of 0.07%. The distribution of errors contained several outlier nucleotides for which the frequency of differences from pNL43 was slightly higher than would be expected from a Poisson distribution in that rarely mismatch errors were present at a level between 1.0% and 2.0%. The approach we used to distinguish authentic minor variants from those that may have resulted from technical error was similar to that used in two previous publications [14 15]: minority variants present in ≥2.0% of sequence reads were considered highly unlikely to represent technical errors. Mutations present at levels between 0.5% and 1.9% had a higher risk of originating from a PCR or sequencing artifact. Therefore, low-prevalence variants occurring at a level of 0.5% or higher at positions for which there was an *a priori* suspicion of a possible mutation such as those at known drug resistance positions were also considered authentic variants.

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Results

Previously Untreated Patients with Virus Samples Containing Transmitted K103N

HIV-1 RT and protease sequences were obtained from 1,334 ARV-naïve patients between August 1998 and December 2007. 40 of the 42 (3.1% of the total) patients with the RT mutation K103N had no other detectable drug-resistance mutations. 24 of these 40 patients had plasma HIV-1 RNA levels ≥4.5 log copies/ml. We identified cryopreserved aliquots of plasma for 17 of the samples with sufficiently high plasma HIV-1 RNA levels. From 13 of these cryopreserved aliquots, we successfully extracted ≥100 cDNA virus templates for UDPS.

Table 1 shows the plasma HIV-1 RNA levels, CD4 counts, RT mutations detected by SGRT, and RT mutations detected by solely by UDPS. The median CD4 count was 326 (range: 3 to 832). The median plasma HIV-1 RNA level was 5.2 log copies/ml. Five patients had plasma HIV-1 RNA levels between 4.5 to 4.9 log copies/ml, four had between 5.0 to 5.6, and another four had ≥5.7 log copies/ml respectively. All virus samples belonged to subtype B. Five of the 13 patients had been infected within the year prior to presentation. The duration of infection for the remaining eight patients was not known. SGRT detected a median of six amino acid differences (range: 4 to 14) from the consensus B sequence. In addition to K103N, the RT sequence from one patient had the accessory NNRTI-resistance mutation P225H.

UDPS confirmed each of the mutations detected by direct PCR sequencing in an unmixed form and 15 of the 19 mutations detected by SGRT as part of an electrophoretic mixture. UDPS detected a median of 6 mutations that were not detected by direct PCR sequencing (range: 0 to 10). The median number of additional silent mutations present in ≥2.0% of sequence reads was 11 (range: 2 to 39) and the median number of residual consensus wildtype amino acids was 1 (range: 0 to 10). The one sample without any additional mutations detectable by UDPS was obtained in a patient undergoing primary HIV-1 infection (PID 22127). That sample, however, did have seven silent minor variants detected only by UDPS.

The polymorphic etravirine-associated mutations V90I and V106I were each detected in one sample. No major etravirine-associated mutations were detected at a level above 0.5%. Five samples had one or more NRTI or NNRTI-resistance mutation including 30062 which had four NRTI-resistance mutations: M184V+L210W+T215Y+219Q and 8048 had one NRTI-resistance mutation K65R and one NNRTI-resistance mutation P225H. No NNRTI-resistance mutations as high as 0.5% with the exception of K103S (a likely K103N revertant) which was detected in 0.6% and 0.9% of reads from samples 8048 and 28010, respectively. The sample from PID 30062 – which contained four NRTI-resistance mutations – also had the following minority protease inhibitor (PI) resistance mutations: M46I (0.8%), I84V (0.9%), and L90M (0.9%) (data not shown). No other minority PI-resistant variants were detected.

Six of the patients with transmitted K103N (16387, 22138, 25590, 27791, 27834, 30074) were treated with a regimen containing ritonavir-boosted atazanavir (n=4) or lopinavir (n=2) in combination with TDF + 3TC, FTC or ddI. These patients experienced complete virological suppression for a median of 3 years (range: 2 to 4 years) through January 2009. Five patients were untreated for a median of 3.5 years (range: 2 to 5 years) through January 2009. One patient (8048) was treated with LPV in combination with d4T + abacavir for seven years but was poorly adherent and eventually developed the LPV resistance mutations (I54V and V82A). One patient (26412) was untreated for 3 months and then lost to follow-up.

NNRTI-Experiencing Patients with Virus Samples Containing Acquired K103N Mutations

Between August 1998 and December 2007, HIV-1 RT and protease sequences were obtained from 1,057 NNRTI-experienced patients. By SGRT, 437 (41%) of these patients had a plasma sample containing K103N obtained while receiving a failing NNRTI-containing regimen

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including 160 patients (15%) whose virus samples exhibited no other major NNRTI-resistance mutations. Of these 160 plasma samples, 32 (20%) had plasma HIV-1 RNA levels ≥4.5 log copies/ml. Cryopreserved aliquots were available for 26 samples. For 20 of these aliquots, we successfully extracted ≥100 cDNA virus templates.

Table 2 shows the plasma HIV-1 RNA levels, CD4 counts, RT mutations identified by SGRT, and RT mutations detected by solely by UDPS. The median CD4 count was 188 (range: 27 to 464). The median plasma HIV-1 RNA level was 4.9 log copies/ml (range 4.5 to 5.7). Eleven patients had plasma HIV-1 RNA levels between 4.5 and 4.9, five had 5.0 to 5.6, and another four had ≥5.7 log copies/ml, respectively. All virus samples belonged to subtype B. Direct PCR sequencing identified a median of 8.5 positions (range: 2 to 15) with differences from consensus B sequence. The accessory NNRTI-resistance mutations P225H (n=3), V108I (n=1), A98G (n=1), and H221Y (n=1) were detected in six of the samples. Twelve samples had established NRTI-resistance mutations including M184V/I in 9 samples and T215F/Y in 6 samples.

UDPS confirmed each of the mutations detected by SGRT in an unmixed form and 29 of the 37 mutations identified by SGRT as part of an electrophoretic mixture. UDPS also uncovered a median of 5.5 mutations that were not detected by direct PCR sequencing (range 0 to 11). A median of eight additional silent mutations (range 1 to 19) were present in ≥2.0% of sequence reads and there was a median of one residual consensus wildtype amino acids (range: 0 to 6). The one sample in which UDPS did not detect any additional mutations had four silent minor variants identified only by UDPS in ≥8.5% of sequence reads.

UDPS detected one or more NNRTI (n=12) or NRTI (n=12) resistance mutation in 14 of 20 samples: 10 samples had both NRTI and NNRTI-resistance mutations, two had NNRTI-resistance mutations alone, and two had NRTI-resistance mutations alone. Among the 12 samples with NNRTI-resistance mutations, seven had a total of 11 major etravirine-resistance mutations including L100I (n=2), K101E (n=2), Y181C (n=2), G190A/S (n=5). Four samples had a total of five accessory etravirine-resistance mutations (V90I, V106I, V179D) including two of the seven with major mutations. Ten of the 12 samples had a total of 14 non-etravirine resistance mutations including V108I (n=6), P225H (n=3), K101N (n=1), Y188C (n=1). Y188F (n=1), H221Y (n=1), P236L (n=1).

In conclusion, significantly more patients with acquired K103N than with transmitted K103N were infected with viruses containing nonpolymorphic etravirine-resistance mutations (7/20 vs. 0/13; p=0.03; Fisher's Exact Test). Furthermore, the overall number of nonpolymorphic etravirine-resistance mutations were significantly higher among those with acquired vs transmitted K103N (13 vs 0 mutations; p=0.02; Wilcoxon Rank Sum Test). However, there was no significant difference in the number of polymorphic etravirine-resistance mutations (V90I, V106I, and V179D) between those with acquired and transmitted K103N (4/20 vs 2/13; p=NS).

Discussion

ARV-resistant viruses cause approximately 12% to 15% of new HIV-1 infections in the United States [5 16 17]. Although the prevalence of transmitted resistance does not appear to have changed much in the past 10 years, the proportion of transmitted viruses with NNRTI resistance has increased [18, 19]. Several investigators have shown that minority NNRTI-resistance variants may decrease the virological response to both initial and salvage NNRTI-containing regimens [20-24].

This study shows that ARV-naïve HIV-1-infected patients in whom SGRT detects the NNRTI-resistance mutation K103N are generally not co-infected with minority variants containing

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other major NNRTI-resistance mutations such as those that confer etravirine resistance. In contrast, in NNRTI-experienced patients in whom SGRT detects only K103N, UDPS often detects additional major NNRTI-resistance mutations.

Consistent with other studies, we found that K103N – particularly K103N alone – was the most common pattern of transmitted genotypic NNRTI resistance, occurring in approximately 3.0% of all new infections. The relative absence of minority drug-resistance variants in ARV-naïve patients with K103N is consistent with the transmission bottleneck that occurs at the time of initial HIV-1 infection [25] and the absence of ongoing selective drug pressure following virus transmission. Nonetheless, minority variants were occasionally present in those with transmitted K103N. For example, PID 8048 had the NRTI-resistance mutation K65R and the accessory NNRTI-resistance mutation P225H and PID 30062 contained the NRTI-resistance mutations M184V, L210W, T215Y, and K219Q.

The presence of multiple additional NNRTI-resistance mutations in patients with acquired K103N is consistent with the low genetic barrier to resistance associated with the first generation NNRTIs. Indeed, patients developing virological failure while receiving an NNRTI often develop multiple independent virus lineages containing different NNRTI-resistance mutations that may not all be detectable by direct PCR sequencing[9, 26, 27]. For example, in patients developing the nevirapine-resistance mutation Y181C, salvage therapy with efavirenz (which retains high levels of activity against viruses with Y181C) is often ineffective because the virus quasispecies in such patients often contains multiple other NNRTI-resistance mutations (such as K103N) at low levels that may not be detectable by SGRT [21, 28, 29].

We performed UDPS only on samples with plasma HIV-1 RNA levels ≥4.5 log copies/ml to ensure that ≥100 viral cDNA templates could be recovered from cryopreserved plasma despite the low efficiency of the recovery process [14]. This restriction probably did not influence our findings in ARV-naïve patients because 60% had plasma HIV-1 RNA levels ≥4.5 log copies/ml. However, this restriction may have influenced our findings in NNRTI-expriencing patients because only 20% had plasma HIV-1 RNA ≥4.5 log copies/ml. The lower RNA levels among NNRTI-treated patients may be a consequence of the ongoing activity of other drugs in the patients' regimens and possibly the incomplete phenotypic resistance associated with K103N alone (at least to efavirenz). Whether NNRTI-treated patients with lower plasma HIV-1 RNA levels would be expected to have fewer or more NNRTI-resistant variants is not known.

The finding that etravirine-resistant minority variants were common in NNRTI-experienced patients receiving etravirine is consistent with the results of the recent clinical trial TMC125-C227 in which patients developing virological failure while receiving an NNRTI-containing regimen were less likely to respond to a salvage regimen containing etravirine than to a salvage regimen containing a ritonavir-boosted PI [30]. Patients in TMC125-227 whose viral sequences had K103N generally responded to therapy with etravirine plus two NRTIs. However, the numbers of patients in that study were insufficient to recommend the use of etravirine rather than a ritonavir-boosted PI in patients with K103N.

Based on previous studies, minority NNRTI-resistant variants present at levels of ≥2.0% appear to be significantly associated with virological failure of an initial nevirapine or efavirenz-containing containing ARV regimen.[23, 24] However, the impact of such minority variants on etravirine therapy has not been studied. Although current recommendations support using a boosted PI for treating patients with transmitted NNRTI resistance[1, 31], our study suggests that further investigation of etravirine for the treatment of patients primarily infected with a K103N-containing virus is warranted.

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Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. US Department of Health and Human Services Panel on Clinical Practices for Treatment of HIV Infection A. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. 2008. (The living document, November, 2008), http://aidsinfo.nih.gov/;
2. Cheung PK, Wynhoven B, Harrigan PR. 2004: which HIV-1 drug resistance mutations are common in clinical practice? AIDS Rev Apr-Jun;2004 6(2):107–116. [PubMed: 15332433]
3. Rhee SY, Liu T, Ravela J, Gonzales MJ, Shafer RW. Distribution of human immunodeficiency virus type 1 protease and reverse transcriptase mutation patterns in 4,183 persons undergoing genotypic resistance testing. Antimicrob Agents Chemother Aug;2004 48(8):3122–3126. [PubMed: 15273130]
4. Wensing AM, van de Vijver DA, Angarano G, et al. Prevalence of drug-resistant HIV-1 variants in untreated individuals in Europe: implications for clinical management. J Infect Dis Sep 15;2005 192 (6):958–966. [PubMed: 16107947]
5. Wheeler, W.; Mahle, K.; Bodnar, U., et al. Antiretroviral drug-resistance mutations and subtypes in drug-naive persons newly diagnosed with HIV-1 Infection, US, March 2003 to October 2006 [abstract 648]. 14th Conference on Retroviruses and Opportunistic Infections; 2007.
6. Vingerhoets J, Azijn H, Fransen E, et al. TMC125 displays a high genetic barrier to the development of resistance: evidence from in vitro selection experiments. J Virol Oct;2005 79(20):12773–12782. [PubMed: 16188980]
7. Bacheler L, Jeffrey S, Hanna G, et al. Genotypic correlates of phenotypic resistance to efavirenz in virus isolates from patients failing nonnucleoside reverse transcriptase inhibitor therapy. J Virol 2001;75:4999–5008. [PubMed: 11333879]
8. Eshleman SH, Becker-Pergola G, Deseyve M, et al. Impact of human immunodeficiency virus type 1 (hiv-1) subtype on women receiving single-dose nevirapine prophylaxis to prevent hiv-1 vertical transmission (hiv network for prevention trials 012 study). J Infect Dis Oct 1;2001 184(7):914–917. [PubMed: 11509999]
9. Kassaye S, Lee E, Kantor R, et al. Drug resistance in plasma and breast milk after single-dose nevirapine in subtype C HIV type 1: population and clonal sequence analysis. AIDS Res Hum Retroviruses Aug; 2007 23(8):1055–1061. [PubMed: 17725424]
10. Shafer RW, Hertogs K, Zolopa AR, et al. High degree of interlaboratory reproducibility of human immunodeficiency virus type 1 protease and reverse transcriptase sequencing of plasma samples from heavily treated patients. J Clin Microbiol 2001;39(4):1522–1529. [PubMed: 11283081]
11. Vingerhoets J, Buelens M, Peeters M, et al. Impact of baseline mutations on the virological response to TMC125 in the phase III clinical trials DUET-1 and DUET-2 [abstract 32]. Antivir Ther 2007;12:S34.
12. Vingerhoets J, Peeters M, Azjin H, et al. An update of the list of NNRTI mutations associated with decreased virological response to etravirine: multivariate analysis on the pooled DUET-1 and DUET-2 clinical trial data [abstract 24]. Antivir Ther 2008;13(Suppl 3):A26.
13. Shafer RW, Schapiro JM. HIV-1 drug resistance mutations: an updated framework for the second decade of HAART. AIDS Rev Apr-Jun;2008 10(2):67–84. [PubMed: 18615118]
14. Mitsuya Y, Varghese V, Wang C, et al. Minority human immunodeficiency virus type 1 variants in antiretroviral-naive persons with reverse transcriptase codon 215 revertant mutations. J Virol Nov; 2008 82(21):10747–10755. [PubMed: 18715933]

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15. Margeridon-Thermet S, Shulman NS, Ahmed A, et al. Ultra-deep pyrosequencing of hepatitis B virus quasispecies from nucleoside and nucleotide reverse-transcriptase inhibitor (NRTI)-treated patients and NRTI-naive patients. J Infect Dis May 1;2009 199(9):1275–1285. [PubMed: 19301976]

16. Weinstock HS, Zaidi I, Heneine W, et al. The epidemiology of antiretroviral drug resistance among drug-naive HIV-1-infected persons in 10 US cities. J Infect Dis Jun 15;2004 189(12):2174–2180. [PubMed: 15181563]

17. Bennett, D.; McCormick, L.; Kline, R., et al. U.S. surveillance of HIV drug resistance at diagnosis using HIV diagnostic sera [Abstract 674]. 12th Conference on Retroviruses and Opportunistic Infections; Boston, MA. February 22 - 25; 2005.

18. Pillay D. Current patterns in the epidemiology of primary HIV drug resistance in North America and Europe. Antivir Ther Oct;2004 9(5):695–702. [PubMed: 15535406]

19. Geretti AM. Epidemiology of antiretroviral drug resistance in drug-naive persons. Curr Opin Infect Dis Feb;2007 20(1):22–32. [PubMed: 17197878]

20. Jourdain G, Ngo-Giang-Huong N, Le Coeur S, et al. Intrapartum exposure to nevirapine and subsequent maternal responses to nevirapine-based antiretroviral therapy. N Engl J Med Jul 15;2004 351(3):229–240. [PubMed: 15247339]

21. Lecossier D, Shulman NS, Morand-Joubert L, et al. Detection of minority populations of HIV-1 expressing the K103N resistance mutation in patients failing nevirapine. J Acquir Immune Defic Syndr Jan 1;2005 38(1):37–42. [PubMed: 15608522]

22. Johnson JA, Li JF, Wei X, et al. Minority HIV-1 drug resistance mutations are present in antiretroviral treatment-naive populations and associate with reduced treatment efficacy. PLoS Med Jul 29;2008 5(7):e158. [PubMed: 18666824]

23. Simen BB, Simons JF, Hullsiek KH, et al. Low-abundance drug-resistant viral variants in chronically HIV-infected, antiretroviral treatment-naive patients significantly impact treatment outcomes. J Infect Dis Mar 1;2009 199(5):693–701. [PubMed: 19210162]

24. Goodman D, Mangot N, McColl D, Miller M, Borroto-Esota K, Svarorovskia E. Pre-existing low-levels of K103N HIV-1 RT mutation above a threshold is associated with virological failure in treatment-naive patients undergoing EFV-containing antiretroviral treatment [abstract 41]. Antivir Ther 2009;14(Suppl1):A43.

25. Keele BF, Giorgi EE, Salazar-Gonzalez JF, et al. Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. Proc Natl Acad Sci U S A May 27;2008 105(21):7552–7557. [PubMed: 18490657]

26. Eshleman SH, Mracna M, Guay LA, et al. Selection and fading of resistance mutations in women and infants receiving nevirapine to prevent HIV-1 vertical transmission (HIVNET 012). AIDS Oct 19;2001 15(15):1951–1957. [PubMed: 11600822]

27. Bacheler LT, Anton ED, Kudish P, et al. Human immunodeficiency virus type 1 mutations selected in patients failing efavirenz combination therapy. Antimicrob Agents Chemother 2000;44(9):2475–2484. [PubMed: 10952598]

28. Shulman NS, Zolopa AR, Passaro DJ, et al. Efavirenz- and adefovir dipivoxil-based salvage therapy in highly treatment-experienced patients: clinical and genotypic predictors of virologic response. J Acquir Immune Defic Syndr 2000;23(3):221–226. [PubMed: 10839657]

29. Casado JL, Moreno A, Hertogs K, Dronda F, Moreno S. Extent and importance of cross-resistance to efavirenz after nevirapine failure. AIDS Res Hum Retroviruses Jul 20;2002 18(11):771–775. [PubMed: 12167268]

30. Ruxrungtham K, Pedro R, Latiff G, et al. Impact of reverse transcriptase resistance on the efficacy of TMC125 (etravirine) with two nucleoside reverse transcriptase inhibitors in protease inhibitor-naive, nonnucleoside reverse transcriptase inhibitor-experienced patients: study TMC125-C227. HIV Med Nov 1;2008 9(10):883–896. [PubMed: 18795960]

31. Oette M, Kaiser R, Daumer M, et al. Primary HIV drug resistance and efficacy of first-line antiretroviral therapy guided by resistance testing. J Acquir Immune Defic Syndr Apr 15;2006 41 (5):573–581. [PubMed: 16652031]

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Table 1

Clinical Characteristics and Ultradeep Pyrosequencing (UDPS) Results in 13 ARV-Naive Patients with HIV-1 Samples with the RT Mutation K103N

PID	Year	Months Infected	CD4 count	HIV-1 RNA	Mutations Detected by Standard Genotypic Resistance Testing[*]	Mutations Detected only by UDPS[*]	ETR Mutations[†]	Other NNRTI Mutations
8048	2000	NA	294	5.6	35I, **103N**, 123E, 177E, 178V, 179I, 211K	48T(10), **65R(1.2)**, 68G(2.1), 90I[*](1.3), 103S(0.6), 135L(5.7), 192N(2.1), 195T (2.6), **225H(3.4)**	(1)	2
30269	2006	<12	449	4.5	**103N,** 175H, 207N, 211K	106I[*](6.9), 123E(23), 162C(27), 179I (31), 196R(2.8)	(1)	0
22138	2004	NA	3	5.7	35I, 53DE, 83K, **103KN**, 122EK, 200KAET	35T(8.5), 123N(10), 142V(33), **221Y (1.3)**, 224K(5.5)	0	1
25590	2005	NA	17	5.7	20R, 35T, 39KAET, 40D, 82R, 98S, **103N**, 122E, 123N, 196E, 200A, 207N, 211Q, 214L	35I(3.6), 39S(4.8), 47F (3.3), 48T(2.5), 50L(2.5), 73N(2.7), 102R(4.1), 103S (1.3), 123E(26), **219Q(1.4)**	0	1
30062	2006	NA	392	4.5	**103N**, 122E, 174RQ, 177E, 178M, 179IV, 211K	**184V(1.0)**, 192N(4.5), 195M(2.2), 204Q (7.2), **210W(1.2)**, **215Y(1.1)**, **219Q(2.5)**	0	0
27834	2006	<12	524	5.7	43E, **103N**, 123E, 135T, 162A, 177E, 200A, 207E	48T(3.0), 60I(9.2), **67N(2.1)**, 177K(8.4), 197R(5.6), 207K(2.2)	0	0
28010	2006	NA	525	4.5	60I, 83K, **103KN**, 169D, 178L, 207E	32R(3.0), 103S(0.9), 122E(3.1), 200A (2.3)	0	1
16387	2003	<12	256	5.2	**103N**, 161PQ, 173EK, 207E, **225H**	35I(9.8), 60I(14), 122E(3.8), 174R(2.3), 212S(6.6), 223R(3.5)	0	0
22127	2004	<3	832	5.2	39A, **103N**, 122E, 142V, 162C, 200I		0	0
22219	2004	NA	607	4.5	**103N**, 122P, 162C, 173E, 200A, 211KR	192N(4.8), 203K(1.2)	0	0
26412	2006	<6	269	4.6	60I, **103N**, 104R, 142VI, 178M, 200A, 211K	35I(27)	0	0

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PID	Year	Months Infected	CD4 count	HIV-1 RNA	Mutations Detected by Standard Genotypic Resistance Testing*	Mutations Detected only by UDPS*	ETR Mutations†	Other NNRTI Mutations
27791	2006	NA	326	5.6	**103N**, 123E, 180V, 192ND, 211K	102R(2.5), 166R(15), 177E(2.5), 177G (2.5), 178V(2.6)	0	0
30074	2006	NA	44	5.7	98S, 102RK, **103KN**, 123E, 177E, 200A, 207K, 211K	20R(3.9), 203K(2.6), 207E(9.6)	0	0

Footnote: Header abbreviations: Months infected – months since HIV seroconversion; CD4 count – cells / μL; HIV-1 RNA – plasma HIV-1 RNA levels in log copies / ml; ETR mutations - V90I, A98G, L100I, K101E/H/P, V106I, E138A, V179D/F/T, Y181C/I/V, G190A/S and M230L [12]; Other NNRTI mutations – Major NNRTI resistance mutations other than K103N and NNRTI resistance mutations.

[*] Mutations in bold are nonpolymorphic RT inhibitor resistance mutations. Underlined mutations are NNRTI-resistance mutations. Mutations with an asterisk are associated with etravirine resistance.

[†] Brackets indicate polymorphic mutations.

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Table 2

Clinical Characteristics and Ultradeep Pyrosequencing (UDPS) Results in 20 NNRTI-Treated Patients with Plasma Samples Containing the RT Mutation K103N

PID	NNRTI	CD4 count	HIV-1 RNA	Mutations detected by direct PCR Sequencing[*]	Mutations detected only by UDPS[*]	ETR mutations[†]	Other NNRTI mutations
6584	EFV	464	5.7	**103NK**, 118IV, 123E, 165IT, 174E, 214L	90I[*](8.2), **101E**[*]**(3.8), 101N(2.4),** 104N (3.1), 179D[*](3.4), **188C(1.5),** 189I(2.3), **190A**[*]**(4.9)**	3 (2)	2
6455	EFV	463	4.5	**74L/V**, 101R, 102Q, **103N,** 135V, 142V, 162A, 174H, **184V**, 211K, **221H/Y**, 234I/L	20R(13), **65R(9.7)**, **67N(2.4)**, **75I(14)**, **100I**[*] **(32)**, **108I(4.8)**, **190A**[*]**(5.4)**, **219N(2.7)**, 224V(5.8), **225H(11.2)**, 228R(5.0)	2 (1)	2
1872	NVP	239	4.5	20R, **41L**, 42A/E, 43K/N, 64K/R, **103N,** 122E, 169D, 179I, **184M/V, 215Y**	36D(18), **181C**[*]**(3.5)**, **190A**[*]**(3.2)**, 196E (6.1), **210W(6.3)**, 237N(5.9)	2	0
26423	EFV	27	5.7	35I, 75LV, **103N**, 121Y, 122E, 135T, 176S, 200E, 211K, **225HP**	62V(1.4), 69N(1.1)**, 74V(8.5)**, **101E**[*]**(4.0)**, 106I[*](1.0), **190S**[*]**(4.8)**, **H221Y(1.1)**	2	1
1838	DLV	91	4.9	20R, 35MI, **67N**, 102Q, **103N**, 122E, 162C, **184V**, 200A, 203K, 207E, **210W**, 211K, **215Y**, 223E	6K(8.6), 53K(2.0), **74V(12)**, 83K(58), **108I (28)**, 111I(3.5), 118I(73), **181C**[*]**(7.0)**, **219E (7.9)**, **236L(1.2)**	1	2
5248	EFV	120	5.3	**103N**, 122E, **184V**, 207E, **225H**	6K(2.7), **74V(13)**, **100I**[*]**(14)**, **108I(2.3)**, **219E(1.1)**	1	1
8350	EFV	261	4.5	**41L**, 101K/Q, **103N 108I/V**, 122E, 135T, 142V, 166R, 207E, **215Y**	**184V(1.2)**, **190S**[*]**(3.1)**, T200A(8.5), **225H (1.2)**	1	1
4817	NVP	445	4.5	28A, 83K, **103N**, 135T, **184V**, 200A, 211K	4T(7.6), **67N(2.3)**, V90I[*](1.9), 142T(3.3), 171Y(20), **188F(5.0)**	(1)	1
8077	NVP	64	5	68G, **103N**, 122E, 162C, 165L, 200A/I/T/V, 204D/E, 207E	47V(4.7), 90I[*](10), 102E(4.4), **184V(0.8)**, 207G(4.0)	(1)	0
16347	EFV	215	4.5	11K/R, 60I, 101Q, **103N**, 122E, 135T, 178M, **184V**, 207E, 211K	20R(2.8), 21I(4.8), 43R(5.9), **108I(3.5)**, 207D(13), **225H (4.5)**	0	2
9651	NVP	335	4.5	35T, 39A, **67N**, 70R, **74I/L**, **98G, 103N**, 122E, 135L, 142I/V, **184V, 215F**/I/S/T, 218D/E, **219Q**	6K(5.3), V60I(29), K64R(3.8), **69N(4.6)**, **108I(7.2),** 196E(3.3), 228H(4.0)	0	1
9666	EFV	87	4.6	**41L**, 75A, **103N**, 123E, 135L/M, 162A, 177E, 211K, **215F**	7I(35), **108I(31)**, 178L(24)**, 184V(30)**	0	1
3901	NVP	82	5.7	35T, **103N**	**41L(4.2)**, 122E(19), 135T(15), **184V(4.8), 210W(1.7), 215Y(7.4)**	0	0

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PID	NNRTI	CD4 count	HIV-1 RNA	Mutations detected by direct PCR Sequencing*	Mutations detected only by UDPS*	ETR mutations†	Other NNRTI mutations
5452	NVP	227	5	62VA, **103N**, 122A, 123E, 135T, 177E, **184I**, 197K, 202V, 211K	I142V(3.4), **184V(7.7)**	0	0
1613	EFV	41	4.8	102K/R, **103N**, 162C, **184V**, 194A/E/S. 200A, **215Y**	10I(5.8), 11R(12)	0	0
3956	EFV	161	5.1	35T, 102Q, **103N**, 122E, 162C, 173Q, 200A, 211K	6K (2.1), 27A (6.8), 39A (2.8), 60I (6.5), 169G (11), 177G (2.0)	0	0
7935	NVP	85	4.5	49R, 67N, 70R, **103N**, 122E, 123N, 135T, 169D, 197P, 200I, 202V, 211K	9T(4.8), 11R (6.5), 39I (9.2)	0	0
8428	NVP	30	5.3	20R, 35LV, **103N**, 122E, 207E, **225H**	32T(7.1), 200S(7.9)	0	0
9650	EFV	247	5.7	**41L**, 98S, **103N**, 135T, 162C, 197E, 211K, 215D	60I (10)	0	0
9905	EFV	322	4.5	20R, 102RK, **103N**, 123E, 135T, 196E		0	0

Footnote: Header abbreviations: NNRTI – at sampling time point; CD4 count – cells / μL; HIV-1 RNA – plasma HIV-1 RNA levels in log copies / ml; ETR mutations - V90I, A98G, L100I, K101E/H/P, V106I, E138A, V179D/F/T, Y181C/I/V, G190A/S and M230L [12]; Other NNRTI mutations – Major NNRTI resistance mutations other than K103N and NNRTI resistance mutations.

[*] Mutations in bold are nonpolymorphic RT inhibitor resistance mutations. Underlined mutations are NNRTI-resistance mutations. Mutations with an asterisk are associated with etravirine resistance.

[†] Brackets indicate polymorphic mutations.