Patterns of Resistance Mutations Selected by Treatment of Human Immunodeficiency Virus Type 1 Infection with Zidovudine, Didanosine, and Nevirapine

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Resistance mutations selected in reverse transcriptase (RT) by incompletely suppressive therapy with combination zidovudine and didanosine with or without nevirapine were identified in 141 human immunodeficiency virus type 1 isolates from peripheral blood mononuclear cells of 57 individuals in the AIDS Clinical Trials Group protocol 241. After prolonged treatment (16–48 weeks), the most common nevirapine-selected mutations were RT 181C (15/30 isolates [50%]), 190A (15/30 [50%]), and 101E (9/30 [30%]). RT 102N and 188L, which individually confer cross-resistance to all nonnucleoside RT inhibitors, were seen in a minority of viruses (6/30 [20%] and 4/30 [13%], respectively). Didanosine-resistance mutations arose rarely. A newly recognized mutation, RT 44D, was selected by the nucleosides. Two distinct zidovudine-resistance mutational patterns were noted. Mutations selected during treatment with zidovudine, didanosine, and nevirapine differed among individuals and changed over time. Resistance testing is necessary to identify which mutations are selected by nevirapine-containing combinations.

Treatment of human immunodeficiency virus type 1 (HIV-1) infection with reverse-transcriptase (RT) inhibitors is often limited by emergence of viral resistance conferred by specific amino acid changes in RT. Although resistance patterns have been defined most clearly in the context of monotherapy, resistance may arise during combination therapy by mutational routes different from those seen during monotherapy [1–3]. For instance, high-level resistance to nevirapine emerges rapidly during monotherapy, conferred most commonly by RT Y181C [4]. However, combination therapy with nevirapine and zidovudine selects for nevirapine-resistance mutations other than 181C [4, 5], possibly because 181C increases susceptibility to zidovudine in viruses also containing zidovudine-resistance mutations [6, 7]. Similarly, the didanosine resistance mutation RT L74V may restore susceptibility to zidovudine for some virus strains containing zidovudine-resistance mutations [8] and is usually absent in patients treated with the combination of zidovudine and didanosine [9].

Current guidelines call for combination therapy with \( \geq 3 \) antiretroviral agents to maximally suppress viral replication and forestall the evolution of resistance [10, 11]. Relatively little is known about resistance patterns of virus from individuals with virologic failure to multidrug regimens, including a nonnucleoside RT inhibitor (NNRTI) and 2 nucleoside RT inhibitors. The combination of zidovudine, didanosine, and nevirapine results in more potent and durable suppression of virus replication in previously antiretroviral drug–naive HIV–1–infected persons [12] than in nucleoside RT inhibitor–experienced individuals, such as those in the AIDS Clinical Trials Group (ACTG) protocol 241 [13]. Indeed, in this trial, which predated real-time virus load monitoring and current therapy guidelines,
concurrent plasma HIV-1 RNA levels remained detectable (>200 copies/mL) in the majority of subjects over the 48 weeks of the trial, although the levels were suppressed below pretreatment baseline levels. Optimal salvage regimens for individuals failing this regimen are unclear.

Information on patterns of viral resistance mutations arising during suboptimal treatment will help to design optimal salvage regimens. Baseline virus load and virologic response in ACTG protocol 241 subjects were previously shown to be associated with baseline genotype at RT codon 215 and other zidovudine resistance–associated codon positions [14]. We now extend the analysis of this trial to describe the dominant resistance mutations in RT of virus isolates selected by the incompletely suppressive study regimens.

Subjects and Methods

Study subjects. ACTG protocol 241 was a 48-week, randomized, double-blind placebo-controlled study comparing combined zidovudine and didanosine to combined zidovudine, didanosine, and nevirapine in subjects with HIV-1 infection and baseline CD4+ T-cell counts <350 cells/mm³ [13]. All had ≥6 months of prior nucleoside analogue therapy (zidovudine, didanosine, or zalcitabine), with a median of 25 months; 33% of subjects had received therapy for ≥36 months. Before enrollment, 97% had received zidovudine, 48% didanosine, and 31% zalcitabine. Virus isolates were obtained from a subset of 57 participants in the virology substudy, which included the 198 subjects at 8 of the 16 trial sites [15]. Isolates were requested from all subjects in the 3-drug arm at all virology substudy sites to determine mutational patterns associated with nevirapine. Isolates were requested at baseline, at week 8, and at the end of the study. For this study, baseline and end-of-study isolates were also requested from subjects in the 2-drug arm whose prior therapy had consisted of zidovudine alone, to compare development of didanosine-resistance mutations between study arms.

Virus isolation. Virus isolates were obtained by using the ACTG quantitative microculture protocol ([16] and G.J.H., V.A.J., D.R.K., et al., unpublished data). Briefly, subject peripheral blood mononuclear cells (PBMC) were incubated at 6 serial 5-fold dilutions with uninfected, phytohemagglutinin (PHA; Difco, Detroit)-stimulated donor PBMC. On day 14, cell-free supernatant from each well was assayed for HIV-1 p24 antigen (HIV-1 p24 ELISA; NEN Life Science Products, Boston). Supernatants from p24-positive wells with the least diluted subject PBMC were combined to form the initial virus isolate stock.

Virus isolation was performed at entry to the study (baseline isolates), at 8 weeks into the study (early-therapy isolates), and at the end of the study (late-therapy isolates; primarily from week 48). Plasma HIV-1 RNA levels were measured by quantitative RT polymerase chain reaction (PCR; Roche Molecular Systems, Alameda, CA) with a lower limit of detection of 200 copies/mL and were available for the same time of virus isolation in 136 of 141 cases. Two early-therapy isolates (from the 3-drug arm) were from subjects with concurrent plasma HIV-1 RNA levels <200 copies/mL. All other isolates were obtained when plasma HIV-1 RNA was detectable, and most of them (96%) were obtained when HIV-1 RNA was >1000 copies/mL.

DNA sequencing. The initial virus stock (1 mL) was cultured with 10⁷ PHA-stimulated uninfected donor PBMC. On day 7, infected cells were harvested and lysed (Puregene, Gentra Systems, Minneapolis). A 1.2-kb DNA fragment of HIV-1 pol was amplified by nested PCR from cell-culture lysates by using conditions and primers as described elsewhere [17]. At the time this work was performed, standard methods were not yet available for amplification from RNA for sequencing. Multiple negative, reagent-only control reactions were performed in each amplification, and sequencing was done only if these were negative.

Sequencing was done by hybridization of the population of PCR product from each isolate to high-density oligonucleotide arrays (Affymetrix GeneChip, Santa Clara, CA) [18]. The PCR products were transcribed to RNA by use of T3 or T7 RNA polymerase (Promega, Madison, WI) and fluorescein-labeled rUTP (Boehringer Mannheim, Indianapolis). Fluorescein-labeled RNA fragments were hybridized to the PRT440 sense and antisense chips and scanned by a confocal laser microscope. DNA sequence, including codons 1–242 of RT, was generated by use of GeneChip 2.0 Rule Algorithm (Affymetrix) and analyzed with Sequencher 3.1 (GeneCodes, Ann Arbor). Cross-contamination with either a laboratory strain or an isolate from another subject was excluded for each sequence [19]. DNA sequences have been submitted to GenBank under the accession numbers AF156033–AF156087, AF166012–AF166083, AF198038–AF198050, and AF198448.

Excellent concordance has been shown between this hybridization method and dideoxynucleotide cycle sequencing [17, 18]. A subset of 49 isolates from this study were sequenced by both methods, and excellent concordance was also seen in these specimens in every codon analyzed for resistance here (listed later), with 1 exception. The frequency of the zidovudine-resistance mutation in RT codon 67 was lower with hybridization than with cycle sequencing (G.J.H., V.A.J., D.R.K., et al., unpublished data). Therefore, the frequency of the codon-67 mutation is reported, but codon 67 was excluded from linkage and longitudinal analyses.

Analysis of RT coding sequence. A total of 141 virus isolates from 57 virology substudy participants was examined for resistance mutations, defined as those causing decreased drug susceptibility in vitro to nucleoside RT inhibitors (M41L, D67N, K70R, L210W, T215Y or F, and K219Q or E for zidovudine; K65R, L74V, and M184V for didanosine; K65R, T69D, L74V, M184V, and T215C for zalcitabine; M184V or I or T for lamivudine; V75T for stavudine; K65R, L74V, Y115F, and M184V for abacavir; and A62V, V75I, F77L, F116Y, and G151M for multinucleoside resistance), nucleotide RT inhibitors (K65R and K70E), or NNRTIs (L74V or I, V75I or L, A98G, L100I, K101E or Q or I, K103N or T or Q or R, V106A or I or L, V108I, E138K, T139I, G141E, V179D or E, Y181C or I, Y188C or L or H, V189I, G190A or E or T or Q, P225H, E233V, P236L, and K238T) [20]. Insertion mutations, such as those between RT codons 69 and 70, that confer multinucleoside resistance [3] are not reliably detected by the sequencing method used here [17].

To detect previously unrecognized resistance mutations, codons were identified in which the same specific change in amino acid composition occurred between baseline and the latest available isolate in ≥2 of the subjects with serial isolates. The net change from...
1 amino acid to the other between baseline and latest available isolate was calculated as the number of subjects with a change in 1 direction (for example, RT M41L) minus the number with a change in the opposite direction (for example, RT L41M) among all subjects with serial isolates.

Analysis of NNRTI-resistance mutations developing in the nevirapine arm included 30 subjects with both early- and late-therapy isolates. Analysis of nucleoside RT inhibitor–resistance mutations included 49 subjects from whom both baseline and late-therapy isolates were obtained (14 in the 2-drug arm and 35 in the 3-drug arm). For longitudinal analysis of changes in zidovudine-resistance mutations, isolates from 55 subjects with >1 isolate were included, using the earliest and latest time point for each subject. Potential linkage among mutations present in ≥4 subjects was analyzed to determine whether a specific variant amino acid at 1 codon position was significantly associated with a specific amino acid at another position. For NNRTI-resistance mutations, analyses were done separately in the 34 early-therapy isolates (for codons 103, 106, 108, 181, and 190) and 37 late-therapy isolates (for codons 101, 103, 181, 188, and 190). For nucleoside RT inhibitor–resistance mutations (in codons 41, 69, 70, 210, 215, and 219), the analysis was done on the last available isolate from each subject (57 isolates). Only isolates that were informative at both codons were used (i.e., isolates with ambiguous calls or with amino acids other than wild type or the specified variant at either codon were not included).

Statistics. χ² and Fisher’s exact tests were used. Reported P values are 2-tailed.

Results

NNRTI-resistance mutations selected in nevirapine-containing arm. Several different NNRTI-resistance mutations were identified in paired early- and late-therapy isolates (figure 1).

Table 1. Changes in NNRTI resistance-conferring mutations during combined therapy with zidovudine, didanosine, and nevirapine.

<table>
<thead>
<tr>
<th>NNRTI resistance pattern</th>
<th>Early-therapy isolates (n = 30)</th>
<th>Late-therapy isolates (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. with NNRTI-resistance mutation(s) in RT codon 181–190 region</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>181C and 190A</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>181C alone</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>190A alone</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>188L alone</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>No. with NNRTI-resistance mutation(s) only outside RT codon 181–190 region</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>No. with no NNRTI resistance mutation</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

NOTE. NNRTI, nonnucleoside reverse-transcriptase (RT) inhibitor.

In early-therapy isolates, the most frequent resistance mutations were RT 190A (8/30 isolates [27%]), 181C (7/30 [23%]), 106A (7/30 [23%]), 103N (6/30 [20%]), and 108I (3/30 [10%]). In late-therapy isolates, the most common mutations were different: RT 181C (15/30 [50%]) and 190A (15/30 [50%]) were most common, followed by 101E (9/30 [30%]), 103N (6/30 [20%]) and 188L (4/30 [13%]). RT 181C, 190A, 101E, and, to a lesser extent, 188L were relatively more common in late- than in early-therapy isolates.

Associations among these NNRTI-resistance mutations were also identified. RT 181C and 190A were frequently seen together in late-therapy isolates (9/30) but not in any early-therapy isolate (table 1). In late-therapy isolates, 181C and 190A were often also present with 101E as a 3d mutation (in 4/9 isolates with 181C/190A). In both early- and late-therapy isolates, 188L was significantly associated with wild-type amino acids in both codons 181 (P = .03) and 190 (P = .047) and did not appear in association with any other major NNRTI-resis-

Figure 1. Nonnucleoside reverse-transcriptase (RT) inhibitor–resistance mutations in subjects in the zidovudine plus didanosine plus nevirapine arm.
tance mutation, including 101E, 103N, 106A, 108I, 181C, or 190A. Any NNRTI-resistance mutation in the RT codon 181–190 region was observed in 17 (57%) early-therapy isolates and 25 (83%) late-therapy isolates.

These changes in patterns of NNRTI-resistance mutations in the RT codon 181–190 region were also evident in longitudinal analyses. Among 7 subjects with 181C in the early-therapy isolate, 2 added 190A and 1 changed to 188L as the sole NNRTI-resistance mutation. Among 8 subjects with 190A in the early-therapy isolate, 3 added 181C and 2 retained 190A as the sole mutation in this region. Both subjects with 188L in the early-therapy isolate retained the 188L alone. Eight subjects had NNRTI-resistance mutations only outside the RT codon 181–190 region in the early-therapy isolate; each of these early isolates had 103N, 106A, or both. Four of these acquired both 181C and 190A, and 1 each acquired 188L, 181C, or 190A; 6 of these 8 had neither 103N nor 106A in the late-therapy isolate. Five subjects had no NNRTI-resistance mutations in the early-therapy isolate. Three of these changed to 190A, and 2 remained wild type at all NNRTI-resistance-associated codons. These findings suggest that, with ongoing incompletely suppressive 3-drug therapy containing nevirapine, selective pressures favor the evolution of virus with an NNRTI-resistance mutation pattern containing 181C/190A or, less commonly, 188L alone. The frequency of 103N, the mutation most often selected by efavirenz, was 20% and did not increase with duration of replication under nevirapine-selective pressure.

**Nucleoside RT inhibitor–resistance mutations selected during therapy.** The majority of isolates from subjects with both baseline and late-therapy isolates had zidovudine-resistance mutations at baseline (figure 2): 43% had 41L, 8% had 67N, 55% had 70R, 22% had 210W, 59% had 215Y, 18% had 215F, and 31% had 219Q. Other baseline nucleoside RT inhibitor–resistance mutations included 69D (associated with zalcitabine resistance) in 14% and 69N (a newly described mutation associated with nucleoside resistance [21]) in 6%. No baseline isolate had other mutations associated with resistance to zalcitabine, didanosine, abacavir, nucleotide RT inhibitors, or multiple nucleosides.

The frequency of specific zidovudine-resistance mutations changed little during the trial period, either across both treatment arms (figure 2A) or within each arm (figure 2B, 2C). No didanosine-resistance mutations were seen in any isolate from the 2-drug arm (figure 2B). Two subjects in the 3-drug arm acquired 74V, 1 acquired 75T, and 1 acquired 184V (figure 2C).

The possibility that a previously unreported mutation might have been selected was evaluated. RT E44D was noted to have the greatest increase in frequency during study therapy, arising in virus isolates from 4 subjects: 3 (8%) of 40 subjects in the 3-drug arm and 1 (7%) of 15 subjects in the 2-drug arm. All other amino acid changes arising in ≥4 subjects were changes known to confer drug resistance (zidovudine-resistance–associated M41L, D67N, L210W, and T215Y and nevirapine–resistance–associated K101E, K103N, Y181C, Y188L, and G190A). RT 44D was acquired on a background of zidovudine-resistance mutations 41L and 215Y in 3 of these 4 subjects. Seven subjects had the variant 44D rather than the wild-type 44E at baseline, in each case in association with 41L and 215Y. Each of these subjects maintained the 44D variant during therapy. These data indicate that 44D was selected during incompletely suppressive nucleoside RT inhibitor therapy, predominantly in a background of 41L and 215Y.

The 2 different zidovudine-resistance mutations at codon 215 (215Y vs. 215F) were associated with distinct patterns of mutations in other codons. RT 215Y was associated with the resistance mutations 41L (89% of isolates with 41L had 215Y, P < .001) and 210W (95%, P = .001), as well as with variant 44D (91%, P = .02), but it was associated with wild-type sequences in codons 70 (85%, P < .01) and 219 (80%, P < .001; figure 3). In contrast, RT 215F was relatively more common in isolates with the zidovudine-resistance mutations 70R (38%,
Figure 3. Composition of reverse-transcriptase codon 215 (open box, 215T; shaded box, 215Y; closed box, 215F) in isolates that contain specified amino acids at zidovudine-resistance mutation sites. For each codon position, the wild-type amino acid (41M, 44E, 70K, 210L, 219K) is shown first. At codon 215, T is wild type, and Y and F are 2 different zidovudine-resistance mutations.

Evolution of zidovudine-resistance mutation patterns was also examined longitudinally, comparing the earliest to the latest available isolates. In the early group, 70R was the most common single zidovudine-resistance mutation (in 10/13 isolates). RT 41L/215Y was the most common pattern in isolates with 2 zidovudine-resistance mutations (9/14). Isolates with 3 zidovudine-resistance mutations most often had either 41L/210W/215Y (9/21) or 70R/215F/219Q (6/21). When this analysis was repeated on the group of latest isolates, the same patterns of mutations emerged (results not shown). When isolates for each subject were compared longitudinally, 8 of 10 subjects with 70R as the single early zidovudine-resistance mutation had changes in codon 70 or showed addition of other zidovudine-resistance mutations. In contrast, the 8 subjects with early 41L/215Y had fewer and more predictable changes: 5 added only 210W, and 4 had no change in their zidovudine-resistance mutations. Longitudinal analysis of codons 215 and 219 suggested the relative stability of 215Y with wild-type 219K and of the double mutant 215F/219Q compared with other combinations of zidovudine-resistance mutations in this region. Only 2 (7%) of 28 isolates with initial 215Y/219K and 1 (13%) of 8 isolates with 215F/219Q changed to a different amino acid combination at these codon positions. Other combinations appeared to be less stable: 7 (70%) of 10 isolates with 215Y/219Q, 215T/219Q, or 215F/219K changed, usually to 215F/219Q.

Association of NNRTI-resistance mutations with nucleoside RT inhibitor–resistance mutations. Linkage of mutations selected by the 2 classes of RT inhibitors, investigated separately for early- and late-therapy isolates, suggested that the choice of NNRTI-resistance mutation evolving during nevirapine selection may be influenced by preexisting nucleoside-resistance mutations. Among early-therapy isolates, the NNRTI mutation 106A was associated with 41L and 44D, respectively, in 7 and 4 of 8 isolates with 106A (P < .01), and 103N was associated with 215Y in 6 of 7 (P < .001). Among late-therapy isolates, 101E was significantly associated with 215Y in 7 of 10 (P < .01).

Discussion

Patterns of mutations conferring RT inhibitor resistance were studied here in a group of NNRTI-naïve, highly nucleoside RT inhibitor–experienced HIV-1–infected individuals treated with incompletely suppressive zidovudine, didanosine, and nevirapine for up to 48 weeks. Results indicate heterogeneity in nevirapine-selected mutations among individuals and over time on the incompletely suppressive regimen, suggesting that resistance testing will be necessary to identify which mutations are selected when a nevirapine-containing triple combination is failing. Some dominant nevirapine-selected genotypes that have been reported to remain susceptible to efavirenz in vitro were observed. This suggests that prospective pilot clinical trials may be warranted to determine whether some patients failing to respond to nevirapine regimens without identifiable cross-re-
The methodological approaches of identifying resistance mutations in PBMC virus isolates by hybridization-based sequencing of a population of PCR products affect the interpretation of results. These studies, which began before sequencing from plasma HIV-1 RNA was standardized, are relevant to clinicians who now can obtain HIV-1 genotyping only from plasma HIV-1 RNA. Genotypic data derived by sequencing PCR products amplified from either HIV-1 RNA circulating in patient plasma or from virus isolate–infected cultured-cell DNA have yielded identical results in subjects with plasma HIV-1 RNA that rebounded during therapy with NNRTIs and nucleoside RT inhibitors [22, 23]. Both sources of PCR products reflect the dominant, actively replicating virus, in contrast to amplification of DNA directly from patient PBMC. PCR products amplified directly from patient PBMC DNA reflect largely an archive of replication-incompetent proviruses with dominant resistance mutations different from those of PCR products amplified from HIV-1 RNA in the plasma of the same blood specimen [24] or an isolate [25]. The dominant replication–competent virus present in vivo is identifiable in either plasma HIV-1 RNA or in PBMC virus isolates. Furthermore, dominant sequences derived from the initial coculture and 1 additional PBMC passage do not differ (authors’ unpublished data).

Sequencing by hybridization has excellent concordance with the commercially available dideoxynucleotide cycle sequencing methods [17, 18]. In a subset of 49 isolates from the current study that were sequenced with both methods, there was excellent concordance in every RT codon relevant for drug resistance (including all NNRTI-resistance mutations) studied here, with the exception of codon 67 (G.J.H., V.A.J., D.R.K., et al., unpublished data). Analysis of codon 67 was limited (see Subjects and Methods section). Minority virus subpopulations are not readily detected by either the method used here or those now available to clinicians. The results of the current study, therefore, indicate the dominant mutational patterns selected in actively replicating virus by the drug regimens under study and suggest approaches to choosing salvage therapy effective against the dominantly selected viruses. Furthermore, associations among different mutations suggest that the dominant bases at different codons are physically linked in viral genomes constituting the majority of the population.

Prior work has shown that nevirapine monotherapy selects the RT Y181C substitution in most individuals. However, 181C was not seen in subjects treated with zidovudine and nevirapine [4, 5]. Similarly, 181C was not seen in subjects with virologic failure to combination zidovudine and delavirdine [26]. This was attributed to the ability of 181C to restore susceptibility to zidovudine when it is introduced into a background of zidovudine-resistant virus [6]. The current study shows, in contrast, that nevirapine resistance emerges often with 181C in a background of zidovudine-resistance mutations during incompletely suppressive therapy with zidovudine, didanosine, and nevirapine. This confirms the findings of an earlier pilot study [22]. The most frequent NNRTI-resistance mutations seen early in this incompletely suppressive therapy were 190A, 181C, 106A, 103N, and 108I. Interestingly, zidovudine-resistance mutations appeared to have some influence on the pattern of NNRTI-resistance mutations seen during early therapy, as shown by the association of 106A with 41L and 103N with 215Y in early-therapy isolates.

It has been thought that failure of 1 currently available NNRTI would lead to broad cross-resistance to the entire class. Indeed, the majority of the dominant nevirapine-selected isolates identified here included mutations expected to confer cross-resistance to delavirdine (103N, 106A, or 181C [27]). However, the 2 single mutations known to confer clinically significant efavirenz resistance, 103N and 188L [23], were dominant in only 27% of early-therapy isolates and 33% of late-therapy isolates. RT 181C alone does not confer cross-resistance to efavirenz [28]. However, the phenotypic effects of 190A and 181C/190A in vitro have not yet been reported [28], and the results of this study cannot exclude the presence of a minority subpopulation with 103N or 188L or a multiple-mutations pattern that may herald poor virologic response to efavirenz. A combination of phenotypic and genotypic testing would be the most thorough approach for excluding cross-resistance to efavirenz; only genotyping was performed here. If these data are confirmed by phenotyping, prospective clinical trials would be needed to determine whether lack of detection of cross-resistance to efavirenz will identify some patients early during virus load rebound on nevirapine who might benefit from efavirenz as a component of a salvage regimen.

The changes in pattern of nevirapine-selected mutations over the duration of incompletely suppressive triple therapy also suggest some biological hypotheses. Most early-therapy isolates with 103N or 106A that lacked NNRTI-resistance mutations in the RT codon 181–190 region acquired mutations in this region with ongoing therapy. Similarly, isolates with no NNRTI-resistance mutations at week 8 usually acquired NNRTI-resistance mutations in the RT codon 181–190 region after prolonged therapy. Isolates that initially developed either 181C or 190A usually kept these mutations and often added the other during ongoing selective drug pressure in vivo. These results suggest that, although early NNRTI-resistance mutations in the RT codon 100–110 region may confer enough resistance to nevirapine to allow initial virus escape, variants with mutations in 181C, 190A, both, or 188L eventually dominate the virus population due to improved replicative capacity or increased drug resistance. The changes in NNRTI-resistance mutations during prolonged failure may also be influenced by mutations selected by other drugs, as suggested by the increasing frequency of 101E, which was associated with 215Y.

RT 188L arose in several subjects and was unique among NNRTI-resistance mutations, because it usually appeared with-
out other NNRTI-resistance mutations. The association of 188L with wild-type codons 181 and 190 was statistically significant \( P < .05 \). Because of the proximity of codon 188 to codons 181 and 190, steric effects or other molecular interactions may limit the stability of 307L with 181C or 190A. Alternatively, the viral replicative capacity or drug resistance profile of virus with 188L as the sole NNRTI-resistance mutation may be sufficient for it to outcompete other variants.

A previously uncharacterized RT inhibitor-selected mutation, RT 44D, was found at baseline in several subjects, was associated only with the zidovudine-selected mutations 41L and 215Y, and increased in frequency during ongoing treatment in both arms. A review of RT sequences of HIV-1 clades in the Los Alamos database showed that only isolates from clade B that also had zidovudine-resistance mutations contained the variant 44D instead of the wild-type 44E. These results suggest that 44D is associated with nucleoside RT inhibitor resistance, consistent with other recent reports [29, 30].

By investigating associations among known nucleoside RT inhibitor–resistance mutations, 2 distinct pathways for acquisition of zidovudine-resistance mutations in vivo were identified. Virus with 215Y accumulated 41L, 210W, and 44D sequentially. The other pattern consists of 215F and 219Q, often on a background of 70R. RT 41L, 44D, and 210W were highly unlikely in a background of 215F/219Q. The reasons for the distinct evolutionary routes require further study but may not be attributable solely to the level of resistance to zidovudine, because virus with 67N/70R/215Y/219Q appears to have a level of reduced susceptibility similar to that with 67N/70R/215F/219Q [31].

The didanosine-resistance mutation RT 74V was observed in only 2 individuals, as reported elsewhere when zidovudine and didanosine were coadministered [1, 9]. Other mutations reported to confer resistance to other nucleoside RT inhibitors did arise rarely. RT 184V and 75T, each seen in 1 subject, have been reported to confer some resistance to didanosine, although each is usually selected by other nucleosides [32, 33]. RT 69N was seen in some of our isolates on a background of 70R/215F/219Q. In vitro studies have shown that RT 69N may confer some degree of multinucleoside resistance [21]. The hybridization-based sequencing used in this study may have led to an underestimation of insertion mutations in the RT codon 69–70 region associated with multinucleoside resistance [3].

In summary, our data suggest important interactions between resistance mutations that arise during incompletely suppressive combination RT inhibitor therapy, which may affect drug susceptibility or replicative capacity and may influence the specific evolutionary routes to multidrug resistance. The single mutations known to confer cross-resistance to efavirenz were not commonly dominant during treatment with zidovudine, didanosine, and nevirapine. A more complete characterization of the genotypic and phenotypic correlates of multidrug treatment failure is needed to optimize salvage regimens.

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References