Mutations in HIV-1 Reverse Transcriptase Potentially Associated with Hypersusceptibility to Nonnucleoside Reverse-Transcriptase Inhibitors: Effect on Response to Efavirenz-Based Therapy in an Urban Observational Cohort

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Background. Hypersusceptibility to nonnucleoside reverse-transcriptase inhibitors (NNRTIs) was described in association with reverse-transcriptase (RT) mutations conferring resistance to nucleoside reverse-transcriptase inhibitors (NRTIs). We evaluated the effect of RT mutations associated with hypersusceptibility to NNRTIs on the response to efavirenz-based therapy.

Methods. We analyzed an observational database of patients for whom highly active antiretroviral therapy failed and who received genotypic resistance testing–guided therapy, either efavirenz or protease inhibitor (PI) based. Study end points were achievement of virus load <80 copies/mL, achievement of virus load <80 copies/mL without rebound to >500 copies/mL, and changes in CD4 cell counts.

Results. The baseline RT mutations M41L, M184V, L210W, and T215Y and the M41L/T215Y and M41L/T215Y/M184V combinations were associated with virological suppression for efavirenz-treated patients, whereas, for PI-treated patients, only the M184V mutation was associated with virological suppression, and the L210W mutation showed a negative correlation; no correlation was found between any mutation and virological response without rebound.

Conclusions. The M41L, M184V, L210W, and T215Y mutations were associated with a better, although transient, virological outcome in patients treated with efavirenz-based regimens.
susceptibility to the NNRTI class of antiretroviral drugs in virus strains harboring RT mutations associated with resistance to NRTIs has been postulated [4]. Resistance to NRTIs is associated with mutations at aa positions 40–75, 115–118, 151, 184, and 210–219 in the RT region. Although mutations conferring resistance to NRTIs—especially those at aa positions 41, 44, 67, 69, 74, 75, 118, 184, 210, 215, and 219—have been found to be strongly associated with hypersusceptibility to NNRTIs [4], the mutational patterns conferring hypersusceptibility to NNRTIs are not clearly defined. Thus, in the absence of a phenotypic resistance test, the genotypic assessment of resistance mutations cannot easily identify hypersusceptibility to NNRTIs. Testing for resistance to antiretroviral drugs has become the standard of care to guide treatment after drug failure [5–7], and 2 types of tests are currently available: genotypic tests and phenotypic tests. Although clinical guidelines recommend the use of resistance testing for the management of treatment after drug failure, they are not prescriptive on the choice between genotypic and phenotypic testing. Genotypic tests are presently being used in many clinical sites as the unique tool to assess drug resistance, but hypersusceptibility to NNRTIs cannot be predicted by the use of genotypic tests. Thus, the association of NRTI mutations and virological outcome in patients receiving NNRTI-based antiretroviral therapy are of particular interest.

Hypersusceptibility to NNRTIs is relatively common: it has been detected in >20% of NRTI-experienced patients [4]. Results obtained in clinical studies show a better virological and immunological response to efavirenz-based therapy in patients carrying virus strains phenotypically hypersusceptible to NNRTIs. In a retrospective cohort analysis, Shulman et al. [8] demonstrated that hypersusceptibility to NNRTIs was associated with an improved virological outcome after 24 weeks of therapy with efavirenz-based salvage regimens. Mellors et al. [9] showed that baseline hypersusceptibility to efavirenz was strongly associated with better virological response to multidrug salvage regimens. Finally, Haubrich et al. [10], in a prospective analysis of a clinical trial cohort, clearly demonstrated that the baseline presence of hypersusceptibility to NNRTIs was associated with a greater reduction of HIV RNA levels and an increase of CD4 cell counts.

However, studies are needed to increase our understanding of the clinical relevance of hypersusceptibility to NNRTIs in routine clinical practice. Most information available to date has come from clinical trials or virological studies. Observational studies may be more representative of the real world, since they include patients underrepresented in clinical trials. Hypersusceptibility to NNRTIs is very common among NRTI-resistant viruses, but hypersusceptibility to NNRTIs cannot be directly defined by identification of specific NRTI mutations. Overall, genotypic testing is routinely used by many clinical centers to guide antiretroviral therapy after drug failure, but there are only a few data available on the association of NRTI mutations and virological response to efavirenz-based regimens. Therefore, studies able to define clinical correlates of NRTI mutations associated with different levels of hypersusceptibility to NNRTIs, in response to NNRTI-based regimens, are of particular interest. Such data are needed, since they can increase our understanding of the possible implications of NRTI mutations in patients receiving NNRTI-based antiretroviral therapy. For these reasons, we examined virological and immunological responses in an unselected population of HIV-infected patients for whom stable antiretroviral therapy failed and who received genotypic resistance testing (GRT)–guided efavirenz-based antiretroviral therapy.

**SUBJECTS AND METHODS**

*Study design, setting, and population of patients.* Subjects for this analysis were enrolled at the National Institute for Infectious Diseases Lazzaro Spallanzani (Rome), which provides care for HIV infection to >3500 patients. The present study concerns an analysis of the database of all patients who underwent GRT after highly active antiretroviral therapy (HAART) failure. The study followed the Clinical Research Guidelines of the National Institute for Infectious Diseases L. Spallanzani. Patients included were tested between June 1999 and March 2002. At the time that GRT was performed, a complete clinical history was obtained, including all laboratory test results, HIV/AIDS-related clinical events, and treatment histories, as well as demographic and behavioral characteristics. After GRT was performed, the patients were prospectively evaluated, to assess the virological and immunological responses to therapy.

A total of 470 patients are included in the database. From the database, 357 patients were recruited into this study, 109 of whom started an efavirenz-based triple-drug combination regimen after GRT was performed and 248 of whom started a protease inhibitor (PI)–based regimen. Patients with previous exposure to NNRTIs were excluded. Patients treated with nevirapine after GRT was performed (n = 15) were also excluded, since nevirapine was rarely used after GRT was performed. Both the efavirenz-based and the PI-based regimens contained 2 or 3 NRTIs. PI-based regimens consisted of a single PI or boosted PIs, according to the best possible regimen indicated by GRT.

*GRT.* Direct sequencing of RT and protease genes was performed by use of the Virosaq (1 and 2) HIV-1 Genotyping kit and the 3100 ABI Sequencer (Applied Biosystems). In brief, RNA was extracted from plasma samples, was retrotranscribed, and was amplified with specific primers. The polymerase chain reaction products covered the whole pol region, coding for all amino acids of the protease and the first 320 aa of RT; that is, the area where the majority of (and the relevant) mutations conferring resistance to antiretrovirals are found.

*Antiretroviral therapy and clinical course.* The results of
Table 1. Characteristics of the 357 study patients at the time of genotypic resistance testing (GRT), according to the antiretroviral regimen assigned after GRT was performed.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All patients (N = 357)</th>
<th>Efavirenz-treated group (n = 109)</th>
<th>PI-treated group (n = 248)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (IQR), years</td>
<td>38 (35–43)</td>
<td>39 (35–44)</td>
<td>38 (34–42)</td>
</tr>
<tr>
<td>Female, no. (%)</td>
<td>109 (30.5)</td>
<td>31 (28.4)</td>
<td>79 (31.9)</td>
</tr>
<tr>
<td>HIV transmission modality, no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intravenous drug use</td>
<td>104 (29.1)</td>
<td>29 (26.6)</td>
<td>75 (30.2)</td>
</tr>
<tr>
<td>MSM</td>
<td>60 (16.8)</td>
<td>15 (13.8)</td>
<td>45 (18.1)</td>
</tr>
<tr>
<td>Heterosexuality</td>
<td>110 (30.8)</td>
<td>40 (36.7)</td>
<td>70 (28.2)</td>
</tr>
<tr>
<td>Other/not reported</td>
<td>73 (23.2)</td>
<td>25 (22.9)</td>
<td>58 (23.4)</td>
</tr>
<tr>
<td>HIV clinical stage, no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDC group A</td>
<td>99 (27.7)</td>
<td>29 (26.6)</td>
<td>70 (28.3)</td>
</tr>
<tr>
<td>CDC group B</td>
<td>125 (35.0)</td>
<td>37 (34.0)</td>
<td>88 (35.5)</td>
</tr>
<tr>
<td>CDC group C</td>
<td>133 (37.3)</td>
<td>43 (39.5)</td>
<td>90 (36.3)</td>
</tr>
<tr>
<td>CD4 cell count at GRT, mean (IQR range), cells/µL</td>
<td>306 (174–469)</td>
<td>357 (189–475)</td>
<td>293 (165–465)</td>
</tr>
<tr>
<td>Lowest previous CD4 cell count, mean (IQR), cells/µL</td>
<td>134 (50–265)</td>
<td>104 (34–254)</td>
<td>153 (54–277)</td>
</tr>
<tr>
<td>Plasma HIV RNA level, mean (IQR), log copies/mL</td>
<td>4.5 (3.9–5.0)</td>
<td>4.6 (4.0–4.9)</td>
<td>4.4 (3.9–5.0)</td>
</tr>
<tr>
<td>Time receiving antiretroviral therapy, mean (IQR), months</td>
<td>39 (27–53)</td>
<td>39 (28–52)</td>
<td>39 (26–54)</td>
</tr>
<tr>
<td>Time receiving HAART, mean (IQR), months</td>
<td>28.5 (16–37)</td>
<td>29.5 (22–37)</td>
<td>27.5 (16–38)</td>
</tr>
<tr>
<td>No. of previous changes of antiretroviral scheme, mean (IQR)</td>
<td>3 (2–5)</td>
<td>3 (2–4)</td>
<td>3 (2–5)</td>
</tr>
</tbody>
</table>

NOTE. *P* values for all characteristics were not significant. CDC, Centers for Disease Control and Prevention; HAART, highly active antiretroviral therapy; IQR, interquartile range; MSM, men who have sex with men; PI, protease inhibitor.

The GRTs were provided by the study virologists through a report containing the list of the mutations for the RT and protease genes, their interpretation, and some considerations of the drugs that can be considered to be either resistant or still active. The patients’ GRT-guided therapy was decided by the treating physician, who took into account both the report on the GRT and the clinical considerations. Patients for whom options for achieving full virological suppression were limited (i.e., those with controversial mutational patterns) and patients harboring multidrug-resistant virus were discussed weekly in a setting that included both the treating physicians and the virologists. These weekly meetings further generated expert advice. In this setting, a final decision was routinely made for the most-controversial cases, taking into account the mutational pattern, the clinical history, the treatment options, and the behavioral and treatment-adherence characteristics of each patient. The subsequent clinical course, including the nature and timing of laboratory testing, was entirely under the responsibility of the treating physician. Clinical and laboratory data were collected and analyzed only while the patients were receiving stable antiretroviral therapy.

**Virological and immunological response to therapy.** Virological suppression was defined as achievement of plasma virus load <80 copies/mL in at least 1 plasma sample during follow-up. Virological suppression without rebound was defined as achievement of plasma virus load <80 copies/mL without virological rebound to >500 copies/mL during the subsequent observation period. For immunological response, we calculated the difference between the CD4 cell count at the time of the last observation and the CD4 count at the time that GRT was performed.

Clinical and laboratory data were collected and analyzed only while the patients were receiving stable antiretroviral therapy. The data at the last observation were defined either as the data at the time when patients experienced any change in antiretroviral therapy, for any reason, or, for patients who did not experience any change in antiretroviral therapy, the last available data during the observation period.

To define the effect of a single NRTI mutation on virological suppression, the statistical association between a set of primary NRTI mutations—including nucleoside-associated mutations, mutations associated with multi-NRTI resistance, and the L74V, V75I, and M184V mutations—and the virological response was analyzed. Moreover, in accordance with recent data [4, 7] indicating an association of NRTI mutations with an increased sensitivity to efavirenz, we analyzed the effect of the M41L/T215Y and M41L/T215Y/M184V combinations.

**Statistical methods.** The statistical analysis included cross-sectional and follow-up analyses. A simple cross-sectional analysis, using the χ² test for discrete variables and analysis of variance (ANOVA) for continuous variables, was performed to assess possible differences between the efavirenz-treated group...
By definition, all patients who received efavirenz-based regimens were NNRTI naive, whereas most patients who received PI-based regimens were PI experienced.

Prevalence of baseline mutations in the HIV-1 RT gene. The baseline prevalence of RT mutations associated with resistance to NRTIs is shown in table 2. In general, no differences were observed between efavirenz-treated and PI-treated patients. However, we observed a significantly high proportion of PI-treated patients carrying the L74V mutation and, conversely, a high proportion of efavirenz-treated patients carrying the M184V and V188I mutations and the M41L/T215Y/M184V combination.

Antiretroviral therapy and follow-up. The overall median follow-up time was 16.5 months (interquartile range [IQR], 12–21 months) in efavirenz-treated patients and 13.5 months (IQR, 7–18 months) in PI-treated patients. During the observation period, efavirenz-treated patients received a median of 8 blood tests (IQR, 4–9 tests), including plasma HIV RNA determination, and PI-treated patients received a median of 5 blood tests (IQR, 3–8 tests).

Virological response to therapy. Among the patients who received efavirenz-based regimens, an undetectable virus load was reached by 69 patients (63%) after a median of 1.5 months (IQR, 1–4 months). Twenty-seven such patients showed a virological rebound to >500 copies/mL after a median of 4 months (IQR, 2–6.5 months).

Among the PI-treated patients, an undetectable virus load was reached by 82 patients (33.1%) after a median of 3 months (IQR, 1–4 months). Seventeen such patients showed a virological rebound to >500 copies/mL after a median of 3.5 months (IQR, 2–6 months).

Virological response to therapy, according to baseline RT mutations. The relationships between virological response and baseline RT mutations, in patients who received efavirenz-based therapy, are shown in table 3. By multivariable analysis, the baseline RT mutations significantly (P < .05) associated with virological suppression among the patients who received efavirenz-based regimens were the following: M41L, M184V, L210W, and T215Y and the M41L/T215Y and M41L/T215Y/M184V combinations.

Table 4 describes the relationships between virological response and baseline RT mutations, in patients who received PI-based therapy. In contrast with the findings for the efavirenz-treated patients, among the PI-treated patients, only the M184V mutation was associated with virological suppression, whereas the L210W mutation was associated with failure to achieve an undetectable virus load (table 4). In both groups, no correlation was found between any mutation and virological suppression without rebound.

Finally, we have also examined the role of the selected NRTI backbone agents in the virological response to therapy. In both groups, no statistically significant differences were found be-
Table 3. Probability of virological suppression and of virological suppression without rebound, in patients \((n = 109)\) treated with efavirenz-based therapy after genotype resistant testing (GRT), according to baseline reverse transcriptase (RT) mutations (multivariable analysis, adjusted by CD4 cell count, plasma HIV-RNA level at the time GRT, time of observation, and no. of HIV RNA tests performed during observation).

<table>
<thead>
<tr>
<th>Mutation(s) in the RT gene</th>
<th>Patients with the mutation(s)</th>
<th>Virological suppression</th>
<th>Virological suppression without rebound</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>AOR 95% CI</td>
</tr>
<tr>
<td>M41L</td>
<td>50 (45.9)</td>
<td>34 (68.0)</td>
<td>16 (32.0)</td>
</tr>
<tr>
<td>E44D</td>
<td>6 (5.5)</td>
<td>4 (66.7)</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td>D67N</td>
<td>36 (33.0)</td>
<td>23 (63.9)</td>
<td>9 (27.3)</td>
</tr>
<tr>
<td>T69D</td>
<td>4 (3.7)</td>
<td>3 (75.0)</td>
<td>1 (25.0)</td>
</tr>
<tr>
<td>K70R</td>
<td>29 (26.6)</td>
<td>17 (58.6)</td>
<td>5 (17.2)</td>
</tr>
<tr>
<td>L74V</td>
<td>1 (0.9)</td>
<td>0 (0.0)</td>
<td>1 (100.0)</td>
</tr>
<tr>
<td>V75I</td>
<td>2 (1.8)</td>
<td>1 (50.0)</td>
<td>1 (50.0)</td>
</tr>
<tr>
<td>V118I</td>
<td>30 (27.5)</td>
<td>21 (70.0)</td>
<td>9 (30.0)</td>
</tr>
<tr>
<td>M184V</td>
<td>73 (67.0)</td>
<td>48 (65.8)</td>
<td>25 (34.2)</td>
</tr>
<tr>
<td>L210W</td>
<td>26 (23.9)</td>
<td>18 (69.2)</td>
<td>8 (30.8)</td>
</tr>
<tr>
<td>T215Y</td>
<td>47 (43.1)</td>
<td>33 (70.0)</td>
<td>14 (29.8)</td>
</tr>
<tr>
<td>K219E</td>
<td>5 (4.6)</td>
<td>3 (60.0)</td>
<td>2 (40.0)</td>
</tr>
<tr>
<td>K219Q</td>
<td>16 (14.7)</td>
<td>9 (56.3)</td>
<td>7 (43.7)</td>
</tr>
<tr>
<td>Q151M</td>
<td>3 (2.8)</td>
<td>1 (33.3)</td>
<td>2 (66.7)</td>
</tr>
<tr>
<td>M41L-T215Y</td>
<td>42 (38.5)</td>
<td>28 (66.7)</td>
<td>14 (33.3)</td>
</tr>
<tr>
<td>M41L-T215Y-M184V</td>
<td>28 (25.7)</td>
<td>17 (60.7)</td>
<td>11 (39.3)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of patients, unless otherwise noted. AOR, adjusted odds ratio; CI, confidence interval.

**Immunological response to therapy.** The changes in CD4 cell counts between baseline and the last observation, in patients who received efavirenz-based regimens, according to the mutations or groups of mutations in the RT gene, are shown in table 5. In general, a higher increase in CD4 count was observed in all patients carrying a single mutation, but a statistically significant increase was seen only in patients with virus carrying the M41L/T215Y/M184V combination. The 35 patients with virus carrying the M41L/T215Y/M184V combination gained a mean (± SD) of 93 (± 206) CD4 cells, whereas those without such combinations lost a mean (± SD) of 58 (± 183) CD4 cells. \(P = .042\).

Among the PI-treated patients, a mean (± SD) increase of 30 (± 181) CD4 cells was seen. The mean (± SD) increase in CD4 cell count, according to baseline mutations in the RT gene, ranged from 89 (± 181) CD4 cells in patients with virus showing the M41L/T215Y/M184V combination to 11 (± 136) CD4 cells in patients with virus showing the M41L/T215Y combination. However, the comparison of changes in CD4 cell count, according to selected mutations or combinations of mutations, was not statistically significant.

**DISCUSSION**

In the present study, we have investigated the relationship between the presence of NRTI mutations and the virological and immunological responses to efavirenz-based regimens in an unselected population of HIV-infected patients who are representative of subjects seen in routine practice and for whom stable antiretroviral therapy failed. The study hypothesis was that the RT mutations, known from previous observations as being strongly associated with hypersusceptibility to NNRTIs [4, 8], would be significantly associated with better virological and immunological response to efavirenz-based therapy. Our data showed a significant correlation of the baseline presence of the M41L, M184V, L210W, and T215Y mutations in the RT gene with an increased probability of achieving undetectable virus loads, for efavirenz-based antiretroviral regimens. The correlation with virological response was seen only with the probability of achieving undetectable virus loads, not with the probability of virus loads remaining undetectable without virological rebound. Moreover, we also found a significant association of the presence of the M41L/T215Y/M184V combination with higher increases in CD4 cell counts, for efavirenz-based therapy. In contrast, in patients who received PI-based regimens, among NRTI mutations associated with hypersuscepti-
### Table 4. Probability of virological suppression and of virological suppression without rebound, in patients (n = 248) treated with protease inhibitor–based therapy after genotype resistant testing (GRT) was performed, according to baseline reverse transcriptase (RT) mutations (multivariable analysis, adjusted by CD4 cell count, plasma HIV-RNA at GRT, time of observation, and number of HIV RNA tests performed during observation).

<table>
<thead>
<tr>
<th>Mutation(s) in the RT gene</th>
<th>Patients with the mutation(s)</th>
<th>Yes</th>
<th>No</th>
<th>AOR 95% CI</th>
<th>Yes</th>
<th>No</th>
<th>AOR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>M41L</td>
<td>92 (37.1)</td>
<td>24 (26.1)</td>
<td>68 (73.9)</td>
<td>0.787</td>
<td>0.411–1.505</td>
<td>22 (23.9)</td>
<td>70 (76.1)</td>
</tr>
<tr>
<td>E44D</td>
<td>25 (10.1)</td>
<td>6 (24.0)</td>
<td>19 (76.0)</td>
<td>0.746</td>
<td>0.233–2.385</td>
<td>6 (24.0)</td>
<td>19 (76.0)</td>
</tr>
<tr>
<td>D67N</td>
<td>82 (33.1)</td>
<td>20 (24.4)</td>
<td>62 (75.6)</td>
<td>0.731</td>
<td>0.358–1.492</td>
<td>18 (22.0)</td>
<td>64 (78.0)</td>
</tr>
<tr>
<td>T69D</td>
<td>19 (7.7)</td>
<td>4 (21.1)</td>
<td>15 (78.9)</td>
<td>0.760</td>
<td>0.182–3.174</td>
<td>4 (21.1)</td>
<td>15 (78.9)</td>
</tr>
<tr>
<td>K70R</td>
<td>58 (23.4)</td>
<td>20 (34.5)</td>
<td>38 (65.5)</td>
<td>1.220</td>
<td>0.552–2.693</td>
<td>13 (22.4)</td>
<td>45 (77.6)</td>
</tr>
<tr>
<td>L74V</td>
<td>20 (8.1)</td>
<td>5 (25.0)</td>
<td>15 (75.0)</td>
<td>0.724</td>
<td>0.386–1.357</td>
<td>4 (20.0)</td>
<td>16 (80.0)</td>
</tr>
<tr>
<td>V75I</td>
<td>4 (1.6)</td>
<td>1 (25.0)</td>
<td>3 (75.0)</td>
<td>4.544</td>
<td>0.220–94.005</td>
<td>1 (25.0)</td>
<td>3 (75.0)</td>
</tr>
<tr>
<td>V118I</td>
<td>40 (16.1)</td>
<td>11 (27.5)</td>
<td>29 (72.5)</td>
<td>0.754</td>
<td>0.315–1.806</td>
<td>7 (17.5)</td>
<td>33 (82.5)</td>
</tr>
<tr>
<td>M184V</td>
<td>127 (51.2)</td>
<td>53 (41.7)</td>
<td>74 (58.3)</td>
<td>2.738</td>
<td>1.482–5.060</td>
<td>42 (33.1)</td>
<td>85 (66.9)</td>
</tr>
<tr>
<td>L210W</td>
<td>60 (24.2)</td>
<td>11 (18.3)</td>
<td>49 (81.7)</td>
<td>0.424</td>
<td>0.183–0.970</td>
<td>13 (21.7)</td>
<td>47 (78.3)</td>
</tr>
<tr>
<td>T215Y</td>
<td>85 (34.3)</td>
<td>23 (27.1)</td>
<td>62 (72.9)</td>
<td>0.868</td>
<td>0.450–1.677</td>
<td>21 (24.7)</td>
<td>64 (73.3)</td>
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<tr>
<td>K219E</td>
<td>13 (5.2)</td>
<td>4 (30.8)</td>
<td>9 (69.2)</td>
<td>0.952</td>
<td>0.244–3.708</td>
<td>2 (15.4)</td>
<td>11 (84.6)</td>
</tr>
<tr>
<td>K219Q</td>
<td>43 (17.3)</td>
<td>14 (32.6)</td>
<td>29 (67.4)</td>
<td>0.949</td>
<td>0.389–2.313</td>
<td>9 (20.9)</td>
<td>34 (79.1)</td>
</tr>
<tr>
<td>Q151M</td>
<td>12 (4.8)</td>
<td>5 (41.7)</td>
<td>7 (58.3)</td>
<td>1.509</td>
<td>0.318–7.117</td>
<td>3 (25.0)</td>
<td>9 (75.0)</td>
</tr>
<tr>
<td>M41L-T215Y</td>
<td>74 (29.8)</td>
<td>18 (24.3)</td>
<td>56 (75.7)</td>
<td>0.716</td>
<td>0.350–1.463</td>
<td>17 (23.0)</td>
<td>57 (77.0)</td>
</tr>
<tr>
<td>M41L-T215Y-M184V</td>
<td>35 (14.1)</td>
<td>11 (31.6)</td>
<td>24 (68.6)</td>
<td>1.453</td>
<td>0.574–3.676</td>
<td>8 (22.8)</td>
<td>27 (77.1)</td>
</tr>
<tr>
<td>All patients</td>
<td>248 (100)</td>
<td>82 (33.1)</td>
<td>166 (66.9)</td>
<td>16.5</td>
<td>0.165–165.0</td>
<td>65 (26.2)</td>
<td>183 (73.8)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of patients, unless otherwise noted. AOR, adjusted odds ratio; CI, confidence interval.

bility to NNRTIs, only the M184V mutation was associated with an increased probability of transiently achieving an undetectable virus load, whereas the remaining mutations showed either no correlation or, as in the case of the L210W mutation, a negative one.

Given the observational nature of our study, we were not able to assess, as Shulman et al. [8] and Haubrich et al. [10] did, the changes in HIV RNA levels by month of study. The relationship at baseline between all single mutations in the RT gene and virological response was investigated by use of a multiple logistic regression model, after adjustment by time of observation, number of blood tests performed during follow-up, CD4 cell counts at the time that GRT was performed, and plasma HIV RNA levels at the time that GRT was performed. In our analysis, we assessed the probability of reaching an undetectable virus load, as an indicator of virological suppression, and the probability of remaining undetectable without rebound to <500 copies/mL during a mean observation period of 16.5 months. This finding is consistent with those of previous studies indicating that, among NNRTI-naive patients for whom HAART has failed, the proportion of patients receiving efavirenz-based regimens who reach an undetectable virus load is ~60% [10, 11].

Our primary objective was to examine whether baseline mutations in the RT gene, associated with hypersusceptibility to NNRTIs, were associated with better virological response to efavirenz-based therapy. We found a significant association of the baseline presence of the M41L, M184V, L210W, and T215Y mutations in the RT gene with an increased probability of achieving undetectable virus load. The baseline presence of such mutations was associated with a 3–4 times higher probability of reaching an undetectable virus load. This finding is consistent with those of previous studies on the topic and reinforces the concept that patients harboring virus hypersusceptible to NNRTIs [10] or with an NRTI mutational pattern associated with hypersusceptibility to efavirenz [8] have a greater reduction of plasma HIV RNA levels while receiving efavirenz-based regimens.

To better understand the association of NRTI mutations with response to antiretroviral therapy, we used the same approach to analyze the virological outcome of patients who, after GRT was performed, received PI-based regimens. In contrast with
the case for patients treated with efavirenz, in the analysis of the virological outcome of patients who received PI-based regimens, only the M184V mutation was associated with increased probability of achieving an undetectable virus load, whereas the remaining mutations in the RT gene showed either no correlation or, as in the case of the L210W mutation, a negative one. The first finding could be explained by the reduced viral fitness and replicative capacity [12] of virus strains harboring the M184V/I mutation. The negative association of the L210W mutation with the response to the PI-based therapy is not surprising, since the L210W mutation is associated with decreased susceptibility to several NRTIs [13, 14] and, thus, could have affected the efficacy of the NRTI class of drugs in the regimen received by our patients.

We also found an absence of any association between baseline NRTI mutations and the probability of maintaining the virological response without virological rebound. This finding is consistent with the observation of Haubrich et al. [10], who, for patients with virus hypersusceptible to NNRTIs, showed a maximum reduction of HIV RNA levels within the first 4 months of NNRTI-based therapy. The association of some NRTI mutations with the probability of achieving undetectable virus load, but not with the probability of virus load remaining undetectable without virological rebound, suggests the need for additional therapeutic strategies to maintain a stable virological response when efavirenz is given to patients who are showing a virological response only if an NNRTI is used in the antiretroviral scheme.

A further aim of our study was to examine whether mutations associated with hypersusceptibility to NNRTIs were also associated with better immunological outcome while receiving efavirenz-based therapy. The relationship between baseline presence of mutations in the RT gene and immunological response was investigated by analyzing changes in CD4 cell counts, according to the mutations or groups of mutations in the RT gene. We found a significant correlation of baseline presence of the M41L/T215Y/M184V combination with increases in CD4 cell counts. The 35 patients with the M41L/T215Y/M184V combination gained a mean of 93 (± 206) CD4 cells, whereas those without such combinations lost a mean of 58 (± 183) CD4 cells (P = .042). This finding is also consistent with those of previous observations in patients receiving NNRTI-based regimens, indicating that patients with virus hypersusceptible to NNRTIs have greater increases in CD4 cell counts, compared with patients with virus with normal susceptibility to NNRTIs [10].

Our study differs from previous studies, in several regards. First, our data were obtained from a cohort of unselected HIV-infected patients seen at a large clinical center for the care of HIV infection. This population sample is representative of all patients who underwent GRT for HAART failure, regardless of social, demographic, and clinical characteristics, and included subjects who generally are underrepresented in clinical trials. Thus, our population sample can be considered to be representative of subjects seen in routine practice. Our results provide support for the association of mutations in the RT gene with hypersusceptibility to NNRTIs and virological and immunological response to efavirenz-based therapy, not only in clinical trials, but also, to our knowledge for the first time, in a population sample representative of the real world. Second, we have also examined the correlation of NRTI mutations associated with hypersusceptibility to NNRTIs with virological response, in patients who received PI-based regimens. Our data indicate that only the M184V mutation was associated with increased probability of transiently achieving an undetectable virus load, whereas the remaining mutations showed either no correlation or, as in the case of the L210W mutation, a negative one—thus reinforcing the concept that mutations associated with hypersusceptibility to NNRTIs are correlated to a better virological response only if an NNRTI is used in the antiretroviral scheme.

Our study has some limitations. A first limitation is the observational nature of our study, which did not allow a detailed evaluation (i.e., by month of observation) of plasma HIV RNA levels and CD4 cell count responses. However, since our data were obtained from a clinical database of patients who received blood tests at different time points, our data analyses seemed to us to be the most reliable methodological approach to evaluate the virological and immunological responses in our population sample. A second limitation is that no phenotypic resistance testing was performed in our study. Thus, the baseline existence of hypersusceptibility to NNRTIs remains a matter of speculation. However, this does not diminish the importance of the association of the baseline NRTI mutations and the virological response to the efavirenz-based therapy. A third limitation is that the virological and immunological responses in

### Table 5. Differences between CD4 cell counts at baseline and those at the last observation, in patients who received efavirenz-based therapy after genotype resistant testing (GRT) was performed, by reverse transcriptase mutations at the time of GRT.

<table>
<thead>
<tr>
<th>Mutation(s)</th>
<th>Mutation present</th>
<th>Mutation absent</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>M41L/T215Y/M184V</td>
<td>+95 ± 177 (50)</td>
<td>+16 ± 230 (59)</td>
<td>.059</td>
</tr>
<tr>
<td>E44D</td>
<td>+173 ± 242 (6)</td>
<td>+66 ± 206 (103)</td>
<td>.151</td>
</tr>
<tr>
<td>M184V</td>
<td>+78 ± 181 (73)</td>
<td>+4 ± 252 (36)</td>
<td>.089</td>
</tr>
<tr>
<td>L210W</td>
<td>+110 ± 191 (26)</td>
<td>+34 ± 213 (83)</td>
<td>.111</td>
</tr>
<tr>
<td>T215Y</td>
<td>+86 ± 163 (47)</td>
<td>+27 ± 238 (62)</td>
<td>.198</td>
</tr>
<tr>
<td>M41L/T215Y</td>
<td>+93 ± 168 (42)</td>
<td>+15 ± 239 (67)</td>
<td>.084</td>
</tr>
<tr>
<td>M41L/T215Y/M184V</td>
<td>+93 ± 206 (28)</td>
<td>−58 ± 183 (81)</td>
<td>.042</td>
</tr>
</tbody>
</table>

**NOTE.** Mean ± SD CD4 cell count change for all patients (n = 109), +53 ± 209 cells/mm³.
the 2 groups were not comparable, since patients in the efa-
virenz-treated group were, by definition, NNRTI naive, whereas
most patients in the PI-treated group were PI experienced.
However, the absence of an association between NNRTI muta-
tions and improved virological and immunological outcomes in
the PI-treated patients could provide indirect support to our
findings for the efavirenz-treated patients.

The results of the study might have important implications
for the optimal management of HIV-infected patients. Several
cohort studies have shown that antiretroviral-therapy failure is
relatively common in routine clinical practice [15, 16]: up to
50% of patients may experience virological failure during their
antiretroviral therapy. Testing for resistance to antiretroviral
drugs has become the standard of care to guide treatment after
drug failure [5–7]. Several studies have shown that genotypic
HIV-1 resistance testing can improve the virological outcome
of therapy for patients for whom antiretroviral therapy has
failed [17, 18]. Expert advice [18, 19], patients’ adherence to
the therapy regimen, and residual therapy options [20] have
been identified as factors associated with better virological
outcome in GRT-guided antiretroviral therapy. The presence of
hypersusceptibility to NNRTIs was also associated with better
virological and immunological outcomes in a prospective clin-
ical trial cohort [10] and in a small retrospective study of pa-
tients receiving salvage regimens [8]. Our data, obtained from
a large clinical database including all patients who received GRT
provides preliminary evidence for the association, in routine
practice, of NNRTI-associated mutations and improved viro-
logical and immunological outcomes in patients receiving efa-
virenz-based therapy.

In conclusion, our data, obtained from an unselected popu-
lation of HIV-infected patients for whom antiretroviral therapy
failed and who are representative of subjects seen in routine
practice, indicate that, among the RT mutations associated with
hypersusceptibility to NNRTIs, the M41L, M184V, L210W, and
T215Y mutations are also associated with better virological and
immunological responses to efavirenz-based antiretroviral ther-
apy. However, a correlation was seen only with the probability
of achieving undetectable virus load, not with the probability of
virus load remaining undetectable without virological rebound.
The latter finding suggests a need for additional therapeutic stra-
tegies to maintain a stable virological response if efavirenz is used
in this setting. The clinical implications of the above-mentioned
benefits on laboratory markers is still a matter of debate.

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