Frequency and Treatment-Related Predictors of Thymidine-Analogue Mutation Patterns in HIV-1 Isolates after Unsuccessful Antiretroviral Therapy

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We investigated, in patients tested between 1991 and 2004, the patterns of mutually exclusive human immunodeficiency virus–1 thymidine-analogue mutations (TAMS) in 4039 reverse-transcriptase sequences with ≥1 TAM. TAM pattern 1, which included M41L and L210W and excluded K70R and F and is coupled with more-extensive cross-resistance to drugs, became the most frequent pattern after 1996. In 1465 genotypes from 684 patients in whom highly active antiretroviral therapy (HAART) was unsuccessful, predictors of this pattern were the number of previous HAART regimens undergone (adjusted odds ratio [OR], 1.09 [95% confidence interval [CI], 1.02–1.16]), use of stavudine/lamivudine (adjusted OR, 1.42 [95% CI, 1.05–1.99]), use of nevirapine (adjusted OR, 1.60 [95% CI, 1.14–2.24]), use of efavirenz (adjusted OR, 1.56 [95% CI, 1.08–2.27]), and use of ritonavir (adjusted OR, 1.35 [95% CI, 1.04–1.75]).

Nucleoside reverse-transcriptase inhibitors (NRTIs) are essential components of highly active antiretroviral therapy (HAART) regimens [1]. Zidovudine and stavudine, 2 thymidine analogues, have been extensively employed in almost all HIV-1–infected patients undergoing therapy. Both agents select for the following group of drug-resistance mutations, called “thymidine-analogue mutations” (TAMS), in the reverse-transcriptase-coding region: M41L, D67N, K70R, L210W, T215Y/F, and K219E/Q [2]. There is strong evidence that TAMs are also responsible for cross-resistance to many other NRTIs [3]. Recently, it has been shown that TAMs tend to cluster into 2 partially distinct patterns—one characterized by the presence of mutations M41L, L210W, and T215Y, leading to more-extensive cross-resistance, and the other containing mutations D67N, K70R, T215F, and K219E/Q, leading to less-extensive cross-resistance [4, 5]. Nonetheless, this distinction is not absolute, because several mutations from one of these groups can coexist with those from the other. In the present study, we sought to classify TAM patterns and to measure their frequency over time, as well as to define the treatment-related determinants of each pattern.

Patients and methods. Patients and sequences for the present study were selected from the Antiretroviral Resistance Cohort Analysis (ARCA) database. ARCA is a public database developed as a tool for the investigation of resistance to drugs used for treatment of HIV: it contains the patients’ treatment data, as well as longitudinally collected CD4 cell counts and data on plasma levels of HIV RNA and on genotypic drug-resistance variations. These data are provided by academic and other public institutions; descriptive statistics can be consulted in the public-access area of the database, at https://www.hivarca.net.

For the present study, we retrieved HIV-1 reverse-transcriptase sequences that contained ≥1 TAM and that were from patients who had received antiretroviral therapy and who had undergone drug-resistance testing between 1 January 1991 and 31 December 2004. To analyze the correlation between treatment-related variables and TAM profiles, we selected the subset of genotypes derived from patients exposed to HAART, defined as use of ≥3 antiretroviral drugs in combination for ≥90 days.

We classified TAM profiles as follows: pattern 1, which in-
cluded either 1 TAM of either 41L, 210W, or 215Y or 2 TAMs (with the wild-type amino acid) at codon 70 and 2 TAMs of 41L and/or 210W; pattern 2, which included either 1 TAM of either 67N, 70R, 215F, or 219E/Q or 2 TAMs (but wild type) at codons 41 and 210; and pattern 3, which included all profiles different from patterns 1 and 2. Revertant mutations at codon 215 [2], as well as the alternative K219N/R mutations, were also considered. In sensitivity analyses, 2 other classification criteria—one of which was derived from the literature [5] and the other of which simply separated into 2 patterns the group of mutations with 215Y and substitutions at codons 41 and 210 versus the group of mutations with 215F and substitutions at codons 67, 70, and 219—were analyzed.

The differences between the frequencies of TAM patterns at any given time and over time were computed by use of the \( \chi^2 \) test and the \( \chi^2 \)-for-trend test, respectively. Determinants of TAM patterns were analyzed by logistic regression. Variables with a \( P < .10 \) in the bivariate analysis were included in multivariate models. All analyses were performed by use of an SPSS software package (version 13.0; SPSS).

**Results.** A total of 4039 reverse-transcriptase sequences containing \( \geq 1 \) TAM were derived from 1802 patients. Of these genotypes, 1465 (from 684 patients) were obtained after the source patient had been exposed to HAART; the median year of genotyping was 2000 (range, 1996–2004). Virus from these patients had been exposed to HAART for a mean of 32 (range, 3–92) months and to NRTIs, non-NRTIs (NNRTIs), and protease inhibitors for a mean of 67 (range, 3–201), 6 (range, 0–78), and 28 (range, 0–92) months, respectively. Before HAART, 67% of these patients had received monotherapy (for 86.4% of the person-years, the monotherapy was with zidovudine), and 81% had received dual-NRTI therapy. The median number of regimens previously used was 5 (range, 1–24), and the median number of drugs used was 6 (range, 3–14). The most frequently employed NRTI-backbone combinations in the treatment history were zidovudine/lamivudine (67.1%), stavudine/lamivudine (57.6%), zidovudine/zalcitabine (48.9%), zidovudine/didanosine (45.3%), stavudine/didanosine (37.2%), didanosine/lamivudine (7.7%), stavudine/abacavir (6.3%), abacavir/lamivudine (5.9%), and didanosine/abacavir (5.1%).

There were 19 TAM profiles with a frequency of \( \geq 1\% \), and they represented 75% of all sequences. The 6 most frequent TAM profiles were 41L/210W/215Y, 41L/67N/210W/215Y, 41L/215Y, 70R, 67N/70R/219Q, and 67N/70R/215F/219Q, and they represented 51% of all sequences. The proportions of isolates belonging to TAM patterns 1, 2, and 3 were 51.2%, 37.2% and 11.7%, respectively. This classification provided the lowest proportion of isolates that were not classifiable as either TAM pattern 1 or TAM pattern 2; when the algorithm proposed by Marcellin et al. [5] was used, a TAM pattern was not assigned to 27% of the sequences, and this proportion increased to as much as 34% when the next separation of all mutations in 2 groups was used.

The frequency of distinct TAM patterns over time is illustrated in figure 1. There was a strong relative increase in the frequency of TAM pattern 1, with a parallel decrease in the frequency of TAM pattern 2 (\( P < .001 \); \( \chi^2 \) test for trend), whereas the frequency of TAM pattern 3 remained stable. Before 1997, TAM pattern 2 was more frequent than TAM pattern 1, whereas the opposite was the case thereafter (\( P < .001 \); \( \chi^2 \) test). The same time trend for the relative proportions of TAM patterns 1 and 2 was observed when the other classification criteria were used (data not shown).

Multiple sequences were available for a subset of 801 patients (median no. of sequences/patient, 3). Longitudinal analysis of this subset, in which the first and last sequences available from each patient were considered, showed that, after unsuccessful antiretroviral therapy, the TAM pattern remained unchanged in 94% of those with TAM pattern 1, in 73% of those with TAM pattern 2, and in 41% of those with TAM pattern 3—and that, indeed, TAM pattern 3 switched to TAM pattern 1 in 47% cases and switched to TAM pattern 2 in 12% of cases.

Bivariate logistic regression showed that each successive calendar year (\( P = .003 \)) and any of the following treatment-related variables were associated with a higher odds ratio (OR) of development of TAM pattern 1: longer time of treatment with dual-NRTI therapy before HAART (\( P = .011 \)), longer time of treatment with HAART (\( P = .006 \)), greater number of drugs and regimens used (both \( P < .001 \)), longer time of treatment with NNRTIs (\( P < .001 \)), and exposures to stavudine (\( P < .001 \)), didanosine (\( P = .005 \)), abacavir (\( P = .002 \)), nevirapine (\( P < .001 \)), efavirenz (\( P < .001 \)), ritonavir (\( P < .001 \)), amprenavir (\( P = .034 \)), and nelfinavir (\( P < .001 \)) and to backbones of stavudine/lamivudine (\( P < .001 \)), stavudine/didanosine (\( P = .002 \)), and stavudine/abacavir (\( P = .019 \)). By contrast, time of treat-
ment with monotherapy (in most cases, zidovudine) was associated with a reduced OR for development of TAM pattern 1 ($P = .003$). Results of multivariate analysis adjusting for all of the aforementioned variables are summarized in table 1; variables independently predictive of TAM pattern 1 were a higher number of failed regimens, previous exposure to nevirapine, efavirenz, or ritonavir, and previous exposure to the NRTI backbone of stavudine/lamivudine; in contrast, longer previous exposure to NRTI monotherapy was independently predictive of a reduced OR for development of TAM pattern 1.

Multivariate analysis showed that a positive predictor of the development of TAM pattern 2 was a longer previous exposure to NRTI monotherapy, whereas negative predictors were a higher number of regimens experienced, a longer previous exposure to dual-NRTI and to NNRTI therapies, and previous exposure to ritonavir and indinavir and to the NRTI backbones of stavudine/lamivudine and stavudine/abacavir (data not shown).

We also analyzed a subset of 391 patients in whom a thymidine analogue–containing HAART was unsuccessful and who had no previous exposure to the alternative thymidinic drug. In this group of 391 patients, there were 343 in whom treatment with zidovudine was unsuccessful and who had no previous exposure to stavudine; of these 343 patients, 44% had TAM pattern 1, 44% had TAM pattern 2, and 12% had TAM pattern 3. The remaining 48 patients in this group of 391 were those in whom treatment with stavudine-containing HAART was unsuccessful; of these 48 patients, 58% had TAM pattern 1, 40% had TAM pattern 2, and 2% had TAM pattern 3. TAM pattern 1 showed a trend toward a higher frequency in the patients in whom treatment with stavudine was unsuccessful ($P = .06$).

Discussion. Analysis of >4000 HIV-1 sequences from treated patients that contained ≥1 TAM showed that, when drug-resistance mutations at codons 41 and 210 were used as the signature of TAM pattern 1 whereas drug-resistance mutations at codon K70R were used as the signature of TAM pattern 2, 88% of all TAM profiles could be classified as belonging to one or the other theses 2 TAM patterns. These criteria are coherent with the ordered appearance of TAMs in vivo [6, 7] and, compared with other criteria, allow classification of more TAM-containing sequences [5]. Indeed, mutations M41L and L210W are rarely selected together with K70R in vivo—in the present study, M41L and L210W were detected in the presence of K70R in only 5.3% and 1.2%, respectively, of the genotypes containing <5 TAMs—probably because the concomitant presence of some of these mutations causes a defect in viral replication capacity [8].

We found that the frequency of TAM pattern 1 increased over time: after 1997, it was approximately twice as frequent as TAM pattern 2. Analysis of viral evolution in patients from whom serial samples were available showed that, in the majority of patients, the initial TAM pathway was retained, particularly in the case of TAM pattern 1. Accumulating clinical evidence has shown that, compared with mutations in TAM pattern 2, those in TAM pattern 1 lead to more-extensive NRTI cross-resistance, with a concomitant loss of drug-treatment options [9–11], which means that the most unfavorable TAM pattern was also the most frequent.

The present study found that, the higher the number of regimens used to treat patients, the higher the probability of development of TAM pattern 1 at treatment failure. Independent of this result, exposure to specific drug regimens also predicted the development of TAM pattern 1 instead of TAM pattern 2; this was found to be the case when patients had previously been treated with NNRTI drugs, and we hypothesize that it could result from continued viral evolution toward NNRTI cross-resistance, which is promoted by the use of a third drug with a low genetic barrier. Also, the use of full-dose ritonavir predicted the development of TAM pattern 1, possibly as a consequence of the lower efficacy of this drug [12]. The combined use of stavudine/lamivudine was independently associated with development of TAM pattern 1, and the use of stavudine/abacavir was negatively associated with development of TAM pattern 2. Notably, these associations were independent of the use of dual-NRTI combinations before HAART. Longer time of previous monotherapy (in most cases, zidovudine) was independently associated with development of TAM pattern 2 instead of TAM pattern 1. Previous studies have consistently shown that K70R, the signature mutation of TAM pattern 2, is often the first TAM to emerge during zidovudine monotherapy [4], whereas zidovudine- or stavudine-containing dual-NRTI therapies do not select for distinct TAM patterns [4, 13, 14]. In the present study, exposure to zidovudine/lamivudine was not associated with the development of TAM pattern 1. Moreover, in a smaller subset of patients—namely, those without previous exposure to thymidine analogues—patients who were being treated with stavudine-containing HAART showed a trend toward a higher prob-

### Table 1. Results of multivariate analysis showing independent association between treatment-related variables and development of thymidine-analogue mutation pattern 1 during failure of highly active antiretroviral therapy (HAART).

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Time on monotherapy (per month longer)</td>
<td>0.99 (0.98–0.99)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Number of regimens</td>
<td>1.09 (1.02–1.16)</td>
<td>.007</td>
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<tr>
<td>Previous treatment</td>
<td></td>
<td></td>
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<tr>
<td>Nevirapine</td>
<td>1.60 (1.14–2.24)</td>
<td>.007</td>
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<tr>
<td>Efavirenz</td>
<td>1.56 (1.08–2.27)</td>
<td>.019</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>1.35 (1.04–1.75)</td>
<td>.026</td>
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<tr>
<td>Stavudine/lamivudine combination</td>
<td>1.42 (1.05–1.92)</td>
<td>.022</td>
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**NOTE.** Other variables in the model were time of treatment with HAART; time of treatment with dual-nucleos(t)ide reverse-transcriptase inhibitor (NRTI) therapy; calendar year of genotyping; time of treatment with non-NRTIs; number of drugs used in treatment; and previous treatment with didanosine, stavudine, abacavir, nelfinavir, amprenavir, and NRTI backbones in HAART (stavudine/didanosine or stavudine/abacavir). CI, confidence interval; OR, odds ratio.
ability of development of TAM pattern 1 at treatment failure than did those who were being treated with zidovudine-containing HAART. Considered together, the results of the present study seem to suggest that, compared with zidovudine-containing regimens, some stavudine-containing regimens are more likely to be associated with the development of TAM pattern 1. Nonetheless, it was also evident that there was not a specific thymidine analogue whose use would preclude development of TAM pattern 1, and therefore this pattern will probably continue to predominate in the future, and a treatment strategy to prevent its development cannot be indicated at present.

One limitation of the present study is its retrospective design, which does not allow analysis of the exact timing of viral evolution under homogeneous drug pressures. Nevertheless, the large number of isolates analyzed, as well as the treatment information available, allowed this difficulty to be at least partly overcome by adjustment of the analysis for all potential confounders that could be identified. A second limitation of the present study is that the majority of viral isolates were from patients who had received either monotherapy or dual therapy before HAART, and caution is therefore advised in any attempt to apply these findings to drug-naive patients starting HAART. Nonetheless, a significant proportion of patients currently receiving HAART, especially those who either have experienced or currently are experiencing failure of treatment for viral infection, are those who have a history of suboptimal outcome of NRTI therapy.

In a large database of HIV-1 isolates from patients who were tested because antiretroviral therapy during 1991–2004 was unsuccessful, the present study found an increased prevalence of TAM pattern 1 over time. In patients in whom a HAART regimen was unsuccessful, this tendency was linked to the failure of multiple regimens, the use of NNRTIs, the use of full-dose ritonavir, and the use of the stavudine/lamivudine combination, whereas a previous history of NRTI monotherapy (in most cases, zidovudine) predicted the emergence of TAM pattern 1 over time. In patients in whom a HAART regimen was unsuccessful, this tendency was linked to the failure of multiple regimens, the use of NNRTIs, the use of full-dose ritonavir, and the use of the stavudine/lamivudine combination, whereas a previous history of NRTI monotherapy (in most cases, zidovudine) predicted the emergence of TAM pattern 1 over time. In patients in whom a HAART regimen was unsuccessful, this tendency was linked to the failure of multiple regimens, the use of NNRTIs, the use of full-dose ritonavir, and the use of the stavudine/lamivudine combination, whereas a previous history of NRTI monotherapy (in most cases, zidovudine) predicted the emergence of TAM pattern 1 over time. In patients in whom a HAART regimen was unsuccessful, this tendency was linked to the failure of multiple regimens, the use of NNRTIs, the use of full-dose ritonavir, and the use of the stavudine/lamivudine combination, whereas a previous history of NRTI monotherapy (in most cases, zidovudine) predicted the emergence of TAM pattern 1 over time. In patients in whom a HAART regimen was unsuccessful, this tendency was linked to the failure of multiple regimens, the use of NNRTIs, the use of full-dose ritonavir, and the use of the stavudine/lamivudine combination, whereas a previous history of NRTI monotherapy (in most cases, zidovudine) predicted the emergence of TAM pattern 1 over time. In patients in whom a HAART regimen was unsuccessful, this tendency was linked to the failure of multiple regimens, the use of NNRTIs, the use of full-dose ritonavir, and the use of the stavudine/lamivudine combination, whereas a previous history of NRTI monotherapy (in most cases, zidovudine) predicted the emergence of TAM pattern 1 over time. In patients in whom a HAART regimen was unsuccessful, this tendency was linked to the failure of multiple regimens, the use of NNRTIs, the use of full-dose ritonavir, and the use of the stavudine/lamivudine combination, whereas a previous history of NRTI monotherapy (in most cases, zidovudine) predicted the emergence of TAM pattern 1 over time. In patients in whom a HAART regimen was unsuccessful, this tendency was linked to the failure of multiple regimens, the use of NNRTIs, the use of full-dose ritonavir, and the use of the stavudine/lamivudine combination, whereas a previous history of NRTI monotherapy (in most cases, zidovudine) predicted the emergence of TAM pattern 1 over time. In patients in whom a HAART regimen was unsuccessful, this tendency was linked to the failure of multiple regimens, the use of NNRTIs, the use of full-dose ritonavir, and the use of the stavudine/lamivudine combination, whereas a previous history of NRTI monotherapy (in most cases, zidovudine) predicted the emergence of TAM pattern 1 over time.