Resistance-Associated Mutations to Etravirine (TMC-125) in Antiretroviral-Naïve Patients Infected with Non-B HIV-1 Subtypes

Almoustapha Issiaka Maïga,1,5,7 Diane Descamps,2 Laurence Morand-Joubert,3 Isabelle Malet,1 Anne Derache,1 Mamadou Cisse,4 Victoria Köta,4 Alain Akonde,5 Bah Diarra,6 Marc Wirden,1 Anatole Tounkara,7 Yvan Verlinden,8 Christine Katlama,1 Dominique Costagliola,9 Bernard Masquelier,10 Vincent Calvez,1 and Anne-Genevieve Marcelin1*

Laboratoire de Virologie, AP-HP, Groupe Hospitalier Pitié-Salpêtrière, UPMC Univ Paris 06, INSERM U943, Paris, France; Laboratoire de Virologie, AP-HP, Groupe Hospitalier Bichat-Claude Bernard, Université Denis Diderot-Paris 7, Paris, France; Laboratoire de Virologie, AP-HP, CHU Saint Antoine, UPMC Univ Paris 06, INSERM U943, Paris, France; CESAC, Bamako, Mali; Solhis, Séguéla, Mali; Laboratoire de Biologie, Hôpital Nianankoro Fomba, Séguéla, Mali; Centre de Recherche et de Formation sur le VIH/TB SEREFO, FMPOS, Université de Bamako, Bamako, Mali; Virco BVBA, Mechelen, Belgium; Service de Maladies Infectieuses, AP-HP, Groupe Hospitalier Pitié-Salpêtrière, UPMC Univ Paris 06, INSERM U943, Paris, France; and Laboratoire de Virologie, CHU de Bordeaux, EA 2968, Université Victor Segalen, Bordeaux, France

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Susceptibility to etravirine (ETR), an expanded-spectrum nonnucleoside reverse transcriptase inhibitor (NNRTI), is dependent on the type and number of NNRTI resistance-associated mutations (RAMs). Studies have shown that some HIV-1 subtypes may have natural polymorphisms described as ETR RAMs. This study addresses the prevalence of ETR RAMs in treatment-naïve patients infected with HIV-1 non-B subtypes and its potential impact on ETR susceptibility. The prevalence of ETR RAMs in 726 antiretroviral-naïve patients infected with non-B HIV-1 subtypes was studied. ETR genotypic resistance was interpreted according to Agence Nationale de Recherches sur le SIDA and Stanford algorithms. NNRTI phenotypic susceptibilities of samples with at least one ETR RAM were measured. Overall, 75 (10.3%) of 726 sequences harbored at least one ETR RAM: sequences from 72 patients (10%) each had one ETR RAM, and sequences from 3 patients (0.4%) each had two ETR RAMs (V90I and Y181C in one case and V90I and A98G in two cases). None of the viruses had three or more ETR RAMs, and none were consequently classified as resistant to ETR. All sequences with two ETR RAMs belonged to subtype CRF02_AG. The presence of one ETR RAM was statistically more frequent in subtype CRF02_AG than in other non-B subtypes (P = 0.004). Three new mutation profiles (E138A and V179I, Y181C and H221Y, and V90I and Y181C) showing decreased ETR phenotypic susceptibility were identified. In conclusion, although the prevalence of ETR RAMs in treatment-naïve patients infected with non-B HIV-1 subtypes was 10%, in most cases this had no significant impact on ETR susceptibility. However, the transmission of drug-resistant viruses with Y181C in a non-B genetic background has a potential for impact on ETR susceptibility.

The efficacy of narrow-spectrum nonnucleoside reverse transcriptase inhibitors (NNRTIs) is limited by the low genetic barrier to resistance, resulting from the relatively easy selection of single mutations that confer nearly complete cross-resistance (16). Etravirine (ETR) is an expanded-spectrum NNRTI with conformational flexibility that allows binding to HIV reverse transcriptase (RT) even in the presence of most mutations that confer resistance to the other NNRTIs (1). In vitro studies showed that multiple mutations are required to confer high-level resistance to the drug (8, 23). In proof-of-principle trials, ETR produced rapid and significant reduction in plasma viral loads in both treatment-naïve patients (10) and those with NNRTI-resistant virus (7, 15, 17). Analyses of the pooled DUET-1 and DUET-2 phase III clinical trial data identified 13 ETR resistance-associated mutations (RAMs) (V90I, A98G, L100I, K101E/P, V106I, V179D/F, Y181C/I/V, and G190A/S). The presence of three or more of these RAMs was associated with decreased virological response to ETR (15, 17). This list was recently updated by adding 4 additional ETR RAMs (K101H, E138A, V179T, and M230L), and a system for weighted scoring has been proposed, with the 17 ETR RAMs having differential weights based upon the impact on response (3.0, Y181I/V, 2.5, L100I, K101P, Y181C, and M230L; 1.5, V106I, V179F, E138A, and G190S; and 1.0, V90I, A98G, K101E/H, V179D/T, and G190A) (25). On the basis of this weighted mutation scoring system, three resulting categories were defined, 0 to 2, 2.5 to 3.5, and 4, corresponding to response rates of 74% (highest response), 52% (intermediate response), and 38% (reduced response), respectively, in the DUET trials. The particularity of HIV-1 is the high level of genetic diversity, caused by errors introduced during the synthesis of cDNA from RNA. HIV-1 is thus subdivided into three groups (M, N,
and O), group M being itself subdivided into nine subtypes (A, B, C, D, F, G, H, J, and K). Subtypes A and F are subdivided into four and two subtypes, respectively: A1, A2, A3, A4, F1, and F2 (21). Genetic recombination between HIV-1 group M subtypes is frequent. Today, 43 circulating recombinant forms (CRFs) have been described and are predominant in Africa and Asia (http://hiv.lanl.gov/content/index). HIV-1 subtypes differ in their structural, regulatory, and accessory genes, long-term repeat sequences, transcriptional promoters, and response to transcriptional factors. These differences may influence susceptibility to antiretroviral drugs (11, 13). In comparisons of subtype B with common non-B subtypes and CRFs, approximately one-half of RT codons are found to be polymorphic in >1% of untreated patients (14). Certain polymorphisms may reduce drug susceptibility, whereas others may facilitate the emergence of major drug resistance during therapy (9).

Some studies have shown that these non-B subtypes may have natural polymorphisms described as ETR RAMs (5, 14). Although ETR previously showed comparable activities against different group M subtypes (A to H), including several CRFs, the testing was done with few strains, and few data concerning the impact of the HIV-1 subtype on the virological response to ETR are currently available (1, 24). The aim of this study was to evaluate the prevalence of ETR RAMs in a large panel of patients who were infected with various non-B HIV-1 subtypes and had never received antiretroviral treatment and to study the ETR phenotypic susceptibilities of the infecting strains.

MATERIALS AND METHODS

Study population. The present study included patients who were infected with non-B HIV-1 subtypes and were monitored in different clinical centers. HIV-1-seropositive individuals were eligible for this study if they had never been exposed to antiretroviral drugs before the time of sampling. Briefly, samples were collected at the time of diagnosis of HIV infection or before the start of antiretroviral treatment. In total, 726 patients from the following centers were included (numbers of patients from each center are indicated in parentheses): CESAC (Centre d’Écoute, de Soins, d’Animation et de Conseils) in Bamako, Mali (163); Nianankoro Fomba Hospital in Segou, Mali (118); Pité-Salpétrière Hospital in Paris, France (192); Bichat-Claude-Bernard Hospital in Paris, France (182); and Saint-Antoine Hospital in Paris, France (71). For each patient, a single HIV-1 sequence was included.

Genotypic resistance analysis and interpretation. In most cases, nucleotide sequence analyses of the pol gene were performed in the participating centers using the centers’ own individual protocols. Samples from CESAC in Bamako and the Nianankoro Fomba Hospital in Segou were sequenced in Paris at the Pité-Salpétrière Hospital. To ensure the quality of the data, each submitted sequence was checked before inclusion. The RT sequences contained at least 15 to 240 amino acids. We studied the prevalence of the following ETR RAMs according to the latest International AIDS Society (IAS)-USA panel list (http://www.iasusa.org; last updated in December 2008): V90I, A98G, L100I, K101E, K101H, K101P, V106I, E138A, V179D, V179F, V179T, Y181C, Y181I, Y181V, G190A, G190S, and M230L. If ETR RAMs were present, results from resistance genotypic tests were interpreted according to the latest versions of Agence Nationale de Recherches sur le SIDA (ANRS) and Stanford algorithms (http://www.hiv.lanl.gov/content/index). CRF_01_cpx, CRF_02_AG, CRF_03_cpx, CRF_04_cpx, CRF_05_cpx, CRF_06_cpx, CRF_07_cpx, CRF_08_cpx, CRF_09_cpx, CRF_10_cpx, CRF_11_cpx, CRF_12_cpx, CRF_13_cpx, CRF_14_BG, H, ADK and URF [unique recombinant form], 59 (8.3%). The distribution of the different non-B subtypes is depicted in Fig. 1A.

Prevalence of ETR RAMs in non-B subtypes. As defined by the December 2008 IAS-USA list of RAMs, 75 (10.3%) of 726 sequences harbored at least one ETR RAM: sequences from 72 patients (10%) each had one ETR RAM, and sequences from 3 patients (0.4%) each had two ETR RAMs (V90I and Y181C in one case and V90I and A98G in two cases). No patient had a sequence with three or more ETR RAMs. According to the latest version of the Stanford list, the mutation V179E was also considered and was present in nine cases (1.2%). The frequencies of all ETR mutations are depicted in Fig. 1B. According to the December 2008 IAS-USA list of RAMs, 75 (10.3%) of 726 sequences harbored at least one ETR RAM: sequences from 72 patients (10%) each had one ETR RAM, and sequences from 3 patients (0.4%) each had two ETR RAMs (V90I and Y181C in one case and V90I and A98G in two cases). No patient had a sequence with three or more ETR RAMs. According to the Stanford list, 88 (12.1%) of 726 sequences harbored at least one ETR RAM: sequences from 84 patients (11.6%) had one ETR RAM, and sequences from 4 patients (0.5%) had two ETR RAMs (V90I and Y181C, E138A and V179E, and in two cases, V90I and A98G). Also, no patient had three or more ETR RAMs (Fig. 1C). All sequences with two or three ETR RAMs belonged to CRF02_AG. The presence of one ETR RAM was statistically more frequent in CRF02_AG than in other non-B subtypes (P = 0.004) (Fig. 1D).

Prevalence of ETR RAMs according to non-B subtype. The distribution of the four ETR RAMs found most frequently in this study by subtype is presented in Fig. 2. V90I was associated with subtypes CRF_02_AG (n = 26; 7%), G (n = 6; 7%), A-1 (n = 2; 5%), and UR (n = 1; 4%) (Fig. 2A). V106I was associated with subtypes D (n = 3; 15%), A-1 (n = 2; 5%), CRF_06 (n = 2; 4%), CRF_01 (n = 1; 5%), and CRF_02 (n = 1; 3%). E138A was associated with CRF_02 (n = 15; 4%), G (n = 2; 2%), C (n = 1; 5%), and CRF_01 (n = 1; 5%) (Fig. 2B). V179E was associated with subtypes CRF02_AG (n = 4; 0.1%), CRF06_cpx (n = 3; 5.8%), CRF14_BG, (n = 1; 50%), and G (n = 1; 1%). The V90I and E138A mutations were associated mainly with the CRF02_AG subtype. Sequences
from two patients had the Y181C mutation, and both patients were infected with CRF02_AG.

**Phenotypic study.** Phenotypic results were available for 20 clinical samples harboring at least one ETR RAM (Table 1). Mutations V90I, A98G, K101E, V106I, and E138A alone were not associated with increased ETR resistance. However, two samples with only one ETR RAM were associated with an increase (3.2-fold) in the ETR IC50. For one sample, which harbored E138A, the ETR IC50 increased 5.2-fold. This sample also contained the RT mutation V179I, which is not an ETR RAM but belongs to the compiled list of mutations associated with resistance to NNRTIs (22). For the other sample, which harbored the Y181C mutation, the ETR IC50 increased 11.1-fold. This sample also contained an H221Y mutation, which is not an ETR RAM but belongs to the compiled list of mutations associated with resistance to NNRTIs (22). Two samples with two ETR RAMs were tested: for the sample with V90I and A98G, the ETR IC50 did not increase (change, 1.2-fold), whereas for the sample with V90I and Y181C, the ETR IC50 increased 3.3-fold. Phenotypic results highlighted that samples with three genotypic profiles, E138A and V179I (from patient no. 1016), Y181C and H221Y (from patient no. 80), and V90I and Y181C (from patient no. 1370), while not considered to be resistant to ETR according to ANRS and Stanford algorithms, corresponded to increases in the ETR IC50 of 5.2-, 11.1-, and 3.3-fold, respectively.

**DISCUSSION**

In this study, the most prevalent non-B HIV-1 subtypes studied were CRF02_AG (55%), G (11%), and CRF06_cpx (7%). Indeed, the majority of the samples studied were from
Mali in West Africa, where the most predominant HIV-1 subtypes are CRF02_AG and CRF06_cpx (5, 6). Other non-B subtypes were found in various but still low proportions.

According to our analysis, most (90%) of the samples from treatment-naïve patients infected with non-B subtypes had no ETR RAMs and no sample was considered to be resistant to ETR (having at least three ETR RAMs) based on genotypic criteria and consideration of mutation patterns (those reported during ETR development or those clinically validated in the DUET trials) (15, 17, 25). Most of the samples with at least one ETR RAM harbored only one ETR RAM (9.8% of all samples). Similar results were found in two previous studies (4, 5). Cotte et al. studied 749 antiretroviral-naïve patients in France and found that although ETR RAMs were present in treatment-naïve patients (around 2%), combinations of RAMs possibly associated with a reduced response to ETR were rare, because sequences harboring ≥3 ETR RAMs or having a weighted ETR genotypic score of ≥2.5 were reported in 0 and 2.3% of cases, respectively. Derache et al. studied 198 sequences from West African treatment-naïve patients and showed that the prevalence of NNRTI primary resistance according to the IAS-USA list of mutations was 9%, due mainly to some ETR-associated mutations (V90I, A98G, V106I) that occur as natural polymorphisms in non-B subtypes. In the study by Derache et al., most of the strains were CRF02_AG, which explains the higher frequency of ETR mutations in this study than in the study by Cotte et al., in which strains were mainly subtype B. In our study, the sequences with one or two ETR RAMs were mainly subtype CRF02_AG, and the most prevalent RAMs as defined by the IAS list were V90I, E138A, and V106I, present in 35 cases (4.4%), 19 cases (2.6%), and 10 cases (1.3%), respectively. Using the definition of the Stanford list, we additionally found V179E in nine cases (1.2%). Phenotypic results showed that for those samples with only one ETR RAM, such as V90I, A98G, K101E, V106I, or E138A, no reduced susceptibility to ETR was observed. This finding is in accordance with the facts...
that mutations V90I, A98G, and K101E have a weight of 1.0 and mutations V106I and E138A have a weight of 1.5 in the ETR scoring system and that, alone, these mutations do not have a significant impact on ETR resistance. However, two samples with one ETR RAM, one with E138A and the other with Y181C, were associated with increases in the ETR IC50. Previous studies suggested that E138A may have a significant impact on ETR resistance, and its presence has been associated with decreased virological response to ETR in multivariate analyses (3, 12, 18). Additionally, the virtual phenotype linear model predicted intermediate changes of 2- to 10-fold in the ETR IC50 for E138A virus (26). Thus, it may be possible that the presence of the E138A mutation alone was responsible for the observed increase in resistance to ETR, but the E138A sample also contained the V179I mutation in the RT gene. Although the V179I mutation is not considered to be an ETR RAM in any of the ETR mutation scores, it has been included in the compiled list of mutations associated with resistance to NNRTIs (22). Consequently, the concomitant presence of both E138A and V179I may be responsible for the observed increase in the IC50 and the impact of V179I, alone and in combination, on ETR resistance should be further investigated. Interestingly, the V179I mutation was also detected in another sample (no. 38) where it was associated with V90I without any impact on the ETR IC50, suggesting that the V179I mutation may have an impact on the ETR IC50 only in combination with some other specific mutations such as E138A.

The other sample with one ETR RAM yielded a high increase in the ETR IC50 (11.1-fold) harbored the Y181C mutation and also H221Y. In vitro studies with Y181C in single-site mutagenesis showed increases in the ETR IC50 of 3.9-fold, but it seems here that the concomitant presence of H221Y, which is not considered to be an ETR RAM, dramatically increases the ETR IC50 (25). Thus, the role of H221Y, alone and in combination, in ETR resistance should be further investigated. Of the samples with two ETR RAMs, only the V90I-Y181C double mutant strain was associated with an increase in the ETR IC50, 3.3-fold, which is just above the cutoff for the assay. Y181C is counted as a high-impact ETR RAM (weight of 2.5), and its presence in combination with at least one other ETR RAM caused, on average, a 12.6-fold reduction in susceptibility to ETR (20, 25). Thus, the observed increase in the ETR IC50 for this sample was probably related mainly to the presence of Y181C and enhanced by the presence of V90I. The sample with V90I and A98G did not show an increase in the ETR IC50, which is not surprising considering that this combination has a weighted ETR score of 2.0.

The presence of the Y181C mutation in two samples (no. 80 and 1370) was due probably to the transmission of drug-resistant viruses from a treated patient to a naïve patient, which is a well-known phenomenon. This is an important point because the transmission of Y181C in a non-B genetic background seems to have a potential impact on ETR susceptibility, as shown by increased ETR IC50 for the V90I-Y181C and Y181C-H221Y profiles.

**Non-B HIV-1 subtypes account for 90% of HIV-1 infections worldwide, and although the HIV-1 group M subtype B is predominant in Europe and the United States, recent studies have indicated that the prevalence of non-B subtypes and CRFs is increasing to significant levels, particularly in immigrants and heterosexually infected individuals (2, 19). In addition, NNRTI-based regimens are used predominantly in resource-poor settings. As some of the ETR RAMs described previously are also recognized as natural polymorphisms in antiretroviral-naïve patients infected with non-B HIV-1 subtypes, it is of particular importance for ETR, which is beginning to be widely used, to be active against all subtypes and groups of HIV-1. It was shown previously in vitro that ETR was active against a series of HIV-1 group M subtypes and CRFs; however, few strains were tested (1). In vivo data from the DUET trials showed that ETR is equally effective in suppressing viral replication in patients infected with HIV-1 subtype B and in patients infected with various non-B HIV-1 subtypes; however, further investigations using HIV-1 clinical isolates from treatment-naïve patients infected with various HIV-1 subtypes are needed (24). In addition, it is not known whether the 10% of subjects with at least one preexisting ETR RAM may be at higher risk of subsequent development of resistance upon treatment with ETR, and this possibility should be further investigated.

In conclusion, non-B HIV-1 subtypes in naïve patients exhibit some naturally occurring ETR RAMs (some of them considered to be medium-impact ETR RAMs). The overall prevalence of ETR RAMs was 10%, and this prevalence had a limited impact on ETR susceptibility. Only three cases were associated with phenotypic resistance to ETR, and in two of three cases, this phenotype was in the context of transmitted Y181C drug resistance. These results confirm those from Andries et al. showing in vitro ETR activities against some non-B HIV-1 strains (1). While these results are very reassuring, they should be confirmed by large in vivo studies analyzing virological responses to ETR for different HIV-1 subtypes.

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