Prevalence of pre-existing resistance-associated mutations to rilpivirine, emtricitabine and tenofovir in antiretroviral-naive patients infected with B and non-B subtype HIV-1 viruses

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Received 22 October 2012; returned 12 December 2012; revised 28 December 2012; accepted 31 December 2012

Objectives: The prevalence of rilpivirine, emtricitabine and tenofovir resistance-associated mutations (RAMs), described in vitro and in vivo, was determined in antiretroviral-naive patients.

Patients and methods: From 2008 to 2011, 1729 treatment-naive patients were tested for resistance by bulk sequencing. We studied the primary rilpivirine RAMs (K101E/P, E138A/G/K/Q/R, V179L, Y181C/I/V, H221Y, F227C and M230I/L) and other potential rilpivirine-associated mutations (V90I, L100I, K101T, E138S, V179D/I, Y188L, V189I, G190A/E/S and M230V). We also studied the M184V/I and K65R mutations for emtricitabine and tenofovir, respectively.

Results: Among 1729 sequences, half of patients had B-subtype viruses and the other half non-B (with 26.7% CRF02, n=461). Primary rilpivirine RAMs were infrequent (4.6%, n=79) and the most prevalent were E138A (3%, n=52), E138K (0.3%, n=5), H221Y (0.3%, n=5), E138G (0.2%, n=4) and Y181C (0.2%, n=4). The frequency of the primary rilpivirine RAMs was similar between B and non-B subtypes. The other potential rilpivirine-associated mutations that were most prevalent were V179I (8.4%, n=145), V90I (3.8%, n=65) and V189I (2.3%, n=40). The common V179I, V189I and V90I polymorphisms have not been associated with virological failure in Phase 3 clinical studies. By the ANRS algorithm, 4.9% (n=84) of samples were resistant to rilpivirine, 3.7% (n=32) of B-subtype viruses versus 6% (n=52) of non-B-subtype viruses (P=0.02, χ² test). The prevalence of K65R and M184I/V was 0.06% (1/1729) and 1% (18/1729), respectively. The prevalence of K103N was 2% (35/1729).

Conclusions: The prevalence of rilpivirine, emtricitabine and tenofovir resistance mutations was very low in antiretroviral-naive patients. The prevalence of resistance to rilpivirine (4.9%, n=84) was not statistically different from the prevalence of efavirenz and nevirapine resistance in our population.

Keywords: primary resistance, non-nucleoside reverse transcriptase inhibitors, NNRTIs

Introduction

Current international,1 European2 and French3 HIV-1 infection treatment guidelines recommend that patients who are symptomatic or who have a low CD4+ cell count receive combination therapy of two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs)4 and a potent third drug from another class, such as a non-nucleoside reverse transcriptase inhibitor (NNRTI), a protease inhibitor or an integrase strand transfer inhibitor. In the USA, treatment is recommended for all HIV-1-infected patients. There is still a need for new NNRTIs with activity against NNRTI-resistant viruses, a higher barrier to the development of resistance and reduced amounts of adverse events.

Indeed, efavirenz and nevirapine are first-generation NNRTIs that are component drugs in many antiretroviral regimens, but they have a relatively low barrier to the development of resistance and exhibit considerable cross-resistance to each other.5
Moreover, they also have a number of associated safety concerns, including hepatotoxicity, neuropsychiatric disorders and cutaneous rash.\textsuperscript{4,6–9}

Etravirine and rilpivirine are second-generation NNRTIs providing alternatives to the commonly used first-generation NNRTI-based regimens.\textsuperscript{5} Etravirine is approved for use in combination with other antiretrovirals in treatment-experienced patients with HIV-1 infection who have experienced previous virological failure with an NNRTI.\textsuperscript{10} Rilpivirine is indicated in the USA in combination with other antiretroviral drugs for the treatment of patients with HIV-1 infection who are antiretroviral-naive,\textsuperscript{11} and in the EU in combination with other antiretroviral drugs for the treatment of patients with HIV-1 infection who are antiretroviral-naive with an HIV-1 RNA level of \( \leq 100000 \) copies/mL.\textsuperscript{12} Rilpivirine was also approved in the USA as a single-tablet regimen with tenofovir disoproxil fumarate and emtricitabine.\textsuperscript{13} Rilpivirine is active in vitro against wild-type viruses and retains activity against most NNRTI-resistant HIV-1 strains, including the most frequently transmitted NNRTI mutation K103N.\textsuperscript{14–16}

Clinical virology data are available from the Phase 2 TMC278-C204 study, a dose-ranging study in HIV-1-infected treatment-naive patients\textsuperscript{17,18} and from two large Phase 3 double-blind studies of efficacy and safety in treatment-naive HIV-infected subjects of 25 mg of rilpivirine once daily and 600 mg of efavirenz once daily in combination with tenofovir/emtricitabine (ECHO) or in combination with two NRTIs (THRIVE).\textsuperscript{19,20}

Based on the available in vitro and clinical data, any of the following amino acid substitutions when present at baseline are likely to reduce the antiviral activity of rilpivirine and are considered primary rilpivirine resistance-associated mutations (RAMs): K101E/P, E138A/G/K/Q/R, V179L, Y181C/I/V, H221Y, F227C and M230I/L. These substitutions are included in the US FDA list of rilpivirine-associated substitutions as noted in the US package insert.\textsuperscript{12,21} Other potential rilpivirine-associated mutations are V90I, L100I, K101T, E138S, V179D/I, Y188L, V189I, G190A/E/S and M230V. Resistance evaluated according to the Agence Nationale de Recherche sur le Sida (ANRS) algorithm was defined as having at least one mutation of K101E/P, E138A/G/K/Q/R/S, V179L, Y181C/I/V, Y188L, H221Y and M230I/L, or having L100I+K103N together (http://www.hivfrenchresistance.org/2011/tab3.html). Resistance to efavirenz and nevirapine was evaluated according to the ANRS algorithm (http://www.hivfrenchresistance.org/2011/tab3.html). Subtype determination was on the basis of the reverse transcriptase and protease coding regions (SmartGene, Switzerland; http://www.smartgene.com).

### Statistical analyses

Between-group comparisons were carried out using Fisher’s exact test for categorical variables and using the \( \chi^2 \) test for continuous variables. The SAS statistical software program version 9.2 was used for analyses.

### Results

#### Distribution of HIV-1 subtypes in antiretroviral-naive patients

Among the 1729 analysed sequences from patients who had never received antiretroviral treatment, 867 were B subtype and 862 non-B subtype. The distribution of non-B subtypes was as follows: CRF02_AG, 461 (26.7%); A1, 59 (3.4%); C, 54 (3.1%); G, 39 (2.3%); D, 34 (2%); CRF01_AE, 34 (2%); CRF06_cpx, 21 (1.2%) and other subtypes, 160 (9.3%) (Table 1).

#### Prevalence of primary rilpivirine RAMs

As defined by the November 2011 International Antiviral Society–USA (IAS-USA) list, the primary rilpivirine RAMs found were rare (4.6%, 79/1729). The most prevalent in this analysis were E138A, in 52 cases (3%); E138K, in 5 cases (0.3%); H221Y, in 5 cases (0.3%); E138G, in 4 cases (0.2%) and Y188L, in 4 cases (0.2%). The frequencies of rilpivirine RAMs are depicted in Figure 1.

### Methods

#### Study population

The present study included 1729 patients who were infected with HIV-1 subtype B (\( n = 867 \)) and subtype non-B (\( n = 862 \)) from university hospitals: Bichat Claude Bernard (Paris), Pitié Salpêtrière (Paris), Pellegrin (Bordeaux) and Saint Antoine (Paris). Samples have been analysed for resistance testing by bulk sequencing from 2008 to 2011. HIV-1 seropositive patients were eligible for this study if they had never been exposed to antiretroviral drugs before the time of sampling. For each patient a single HIV-1 sequence was included in this analysis; we choose the first available, closest to diagnosis if more sequences were available for one patient.

### Genotypic resistance analyses and interpretation

Bulk sequences of the reverse transcriptase genes on RNA were determined in each laboratory using the ANRS consensus technique primer sequences described at http://www.hivfrenchresistance.org. Primary rilpivirine RAMs were defined as K101E/P, E138A/G/K/Q/R, V179L, Y181C/I/V, H221Y, F227C and M230I/L.\textsuperscript{21} The other potentially rilpivirine-associated substitutions studied were V90I, L100I, K101T, E138S, V179D/I, Y188L, V189I, G190A/E/S and M230V. Resistance evaluated according to the ANRS algorithm was defined as having at least one mutation of K101E/P, E138A/G/K/Q/R/S, V179L, Y181C/I/V, Y188L, H221Y and M230I/L, or having L100I+K103N together (http://www.hivfrenchresistance.org/2011/tab3.html). Resistance to efavirenz and nevirapine was evaluated according to the ANRS algorithm (http://www.hivfrenchresistance.org/2011/tab3.html). Subtype determination was on the basis of the reverse transcriptase and protease coding regions (SmartGene, Switzerland; http://www.smartgene.com).

### Table 1. Distribution of HIV-1 subtypes (\( n = 1729 \))

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Number of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>867 (50.1)</td>
</tr>
<tr>
<td>CRF02_AG</td>
<td>461 (26.7)</td>
</tr>
<tr>
<td>A1</td>
<td>59 (3.4)</td>
</tr>
<tr>
<td>C</td>
<td>54 (3.1)</td>
</tr>
<tr>
<td>G</td>
<td>39 (2.3)</td>
</tr>
<tr>
<td>D</td>
<td>34 (1.9)</td>
</tr>
<tr>
<td>CRF01</td>
<td>34 (1.9)</td>
</tr>
<tr>
<td>CRF06</td>
<td>21 (1.2)</td>
</tr>
<tr>
<td>Others</td>
<td>160 (9.3)</td>
</tr>
</tbody>
</table>
In B-subtype viruses the rilpivirine RAMs found to be most prevalent in this analysis were E138A, in 17 cases (2%); E138G, in 3 cases (0.3%); E138K, in 3 cases (0.3%) and H221Y, in 2 cases (0.2%). In non-B-subtype viruses, the rilpivirine RAMs found to be most prevalent were E138A, in 35 cases (4.1%); Y181C, in 3 cases (0.3%); H221Y, in 3 cases (0.3%); K101E, in 2 cases (0.2%); E138K, in 2 cases (0.2%) and M230I, in 2 cases (0.2%).

Using the IAS-USA list of November 2011 for the analysis, most viruses with a primary rilpivirine RAM had only one RAM: 71 sequences (4.1%) had one rilpivirine mutation and 5 sequences (0.3%) had two rilpivirine mutations. No patient had a sequence with three or more rilpivirine RAMs (Figure 2). The distribution of the number of rilpivirine RAMs was significantly different between B- and non-B-subtype viruses (Fisher’s test, \( P = 0.01 \)). Indeed non-B-subtype viruses had more rilpivirine RAMs than B-subtype viruses. This difference is due mainly to the mutation E138A, which was twice as common among non-B subtypes.

Prevalence of other potential rilpivirine-associated mutations

The other potential rilpivirine-associated mutations found to be most prevalent in this analysis were V179I in 145 cases (8.4%), V90I in 65 cases (3.8%) and V189I in 40 cases (2.3%). The frequencies of other rilpivirine mutations are depicted in Figure 1. In B-subtype viruses, the mutations found to be most prevalent in this analysis were V179I in 50 cases (5.8%) and V90I in 41 cases (4.7%). In non-B-subtype viruses, the mutations found to be most prevalent in this analysis were V179I in 95 cases (11%), V189I in 26 cases (3%) and V90I in 24 cases (2.8%).

Among the 1729 sequences, 344 (19.9%) harboured at least one rilpivirine mutation (rilpivirine RAMs plus other rilpivirine mutations): 295 sequences (17%) had one rilpivirine mutation, 44 sequences (2.5%) had two rilpivirine mutations, 2 sequences (0.12%) had three rilpivirine mutations and 3 sequences (0.17%) four rilpivirine mutations (Figure 3). The distribution of the number of rilpivirine mutations (rilpivirine RAMs plus other rilpivirine mutations) was significantly different between the B and non-B subtypes (Fisher’s test, \( P = 0.0255 \)) with more rilpivirine RAMs and other rilpivirine mutations in non-B-subtype viruses. This difference is due mainly to the polymorphic mutation as V179I, the primary rilpivirine RAM E138A and the other NNRTI-associated mutation V189I, all of which were twice as common among non-B subtypes.
Resistance

According to the ANRS algorithm, 4.9% of samples were resistant to rilpivirine, 3.7% of B-subtype viruses versus 6% of non-B-subtype viruses \( (P=0.02, \chi^2 \text{ test}) \). The prevalence of the combination L100I+K103N was 3/1729 (0.17%) and L100I was present only in association with K103N; whereas K103N alone was present in 35 cases (2%). The prevalence of the tenofovir mutation K65R and the emtricitabine/lamivudine mutations M184I/V were 0.06% (1/1729) and 1% (18/1729), respectively, and there was no difference between B and non-B subtypes. According to the ANRS algorithm, resistance to efavirenz and nevirapine in our population was 3.7% and 3.8%, respectively.

Discussion

Our study was the first evaluating the prevalence of primary rilpivirine RAMs and other potential rilpivirine-associated mutations in antiretroviral-naive HIV-1-infected patients \((n=1729)\) and comparing the prevalence of these mutations between B-subtype and non-B-subtype viruses. According to our analysis using both the IAS and ANRS lists, most (95%) of the samples from treatment-naive patients had no primary rilpivirine RAMs and the most prevalent primary rilpivirine RAM was E138A in 3%, which is similar to the Vingerhoets et al.\(^{23}\) study. If we expanded our analysis to include the other potential rilpivirine-associated mutations, the percentage of samples from treatment-naive patients with no rilpivirine mutations fell to 81%. Of the other potential rilpivirine mutations, the most prevalent were polymorphic V179I (8%) and V90I (3.8%). The prevalence of V179I is increased in non-B subtypes (11%), whereas the prevalence of V90I is increased in B-subtype viruses (4.7%). The mutation V179I was also found in HIV-1 mutant strains selected in vitro in the presence of rilpivirine.\(^{14}\) On its own, the mutation V179I does not induce a reduced susceptibility to rilpivirine (<2-fold; antivirogram assay with a cut-off of 2.7), but the mutation V179I can induce an increase of rilpivirine resistance when it is present in combination with other mutations such as Y181C or L100I+Y181C.\(^{14,22}\) Moreover, the Phase 3 ECHO and THRIVE studies showed that V179I and V90I mutations could be treatment emergent, although they were present in a low proportion of rilpivirine virological failures (3%–8%).\(^{22}\) Moreover, the V179I and V90I mutations only conferred phenotypic resistance to rilpivirine when present in combination with primary rilpivirine RAMs.\(^{22}\) The common V179I, V189I and V90I polymorphisms have not been associated with an increased risk of virological failure in Phase 3 clinical studies.\(^{23,24}\)

In the present study, the most prevalent primary rilpivirine RAM was E138A (3%), and this prevalence is more important in non-B-subtype viruses (4.1%). In vitro a site-directed mutant harbouring E138A alone presented a fold change in the antiretroviral activity of rilpivirine of 2.5.\(^{14}\) In vivo, it has been shown that E138A is a RAM associated with a decrease in susceptibility to rilpivirine.\(^{22}\) Moreover, Haddad et al.\(^{25}\) showed that the reduction in rilpivirine susceptibility caused by E138A was found to be similar (with a fold change of 1.9 in the PhenoSense assay with a cut-off of 2.0) to that observed with the primary rilpivirine RAMs E138G, K and Q. Several studies have shown the E138A mutation to be an etravirine RAM.\(^{26,27}\) A German study also showed a low prevalence of three studied NNRTI RAMs (E138K, Y181I/V and K101E), but their population comprised pre-treated patients.\(^{28}\)

Overall, the prevalence of resistance to rilpivirine in our study was 4.9%, according to the ANRS resistance algorithm. This prevalence was not statistically different from efavirenz and nevirapine resistance in our population: 3.7% and 3.8%, respectively. The prevalence of the primary rilpivirine RAMs determined here was consistent with the prevalence of that of NNRTI resistance mutations from the Odyssee study, where 2.4% of samples had greater than or equal to one primary NNRTI mutation.\(^{29}\) The prevalence of rilpivirine, emtricitabine/lamivudine and tenofovir resistance mutations was very low in antiretroviral-naive patients. However, resistance testing should be considered before initiation of NNRTI-based treatment in antiretroviral-naive patients, as recommended in the EU Summary of Product Characteristics.

Figure 3. Number of rilpivirine mutations (primary RAMs plus other potential mutations).
Rilpivirine resistance in antiretroviral-naive patients

Acknowledgements
We thank G. Le Molliier and P. Grange for their technical assistance.

Funding
This work was supported by the Agence Nationale de Recherches sur le SIDA (ANRS), the European Community’s Seventh Framework Programme (FP7/2007-2013) under the project ‘Collaborative HIV and Anti-HIV Drug Resistance Network (CHAIN)’ (grant agreement no. 223131) and the ARVD (Association de Recherche en Virologie et Dermatologie).

Transparency declarations
None to declare.

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