The HIV-1 integrase G118R mutation confers raltegravir resistance to the CRF02_AG HIV-1 subtype

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Background: Most of the previous studies that explored the molecular basis of raltegravir resistance were conducted studying the HIV-1 B subtype. It has been shown that the CRF02_AG subtype in relation to its natural integrase (IN) sequence could develop different genetic pathways associated with raltegravir resistance. The aim of this study was to explore resistance pathways preferably used by CRF02_AG viruses compared with subtype B.

Methods: Twenty-five HIV-1 CRF02_AG-infected patients failing a raltegravir-containing regimen were studied. IN gene sequences were examined for the presence of previously described IN inhibitor (raltegravir, elvitegravir, dolutegravir and MK-2048) resistance mutations at 20 amino acid positions.

Results: Among the 25 studied patients, 7 showed viruses harbouring major raltegravir resistance mutations mainly associated with the 155 genetic pathways and 18 showed viruses harbouring none of them; however, for 1 patient, we found a 118R mutation, associated with MK-2048 in vitro resistance, in a 74M background. For this patient, the phenotypic analysis showed that addition of only the G118R mutation conferred a high level of resistance to raltegravir (fold change = 25.5) and elvitegravir (fold change = 9.2).

Conclusions: This study confirmed that mutation pathways for raltegravir resistance could be different between the two subtypes CRF02_AG and B with a preferential use of the 155 mutation in non-B subtypes. A new genetic pathway associated with raltegravir resistance, including the 118R mutation, has also been identified. This new genetic pathway, never described in subtype B, should be further evaluated for phenotypic susceptibility to dolutegravir and MK-2048.

Keywords: integrase inhibitors, MK-2048, phenotype

Introduction

Raltegravir and elvitegravir are the first members of a new class of HIV-1 integrase inhibitors (INIs) interfering with the integrase (IN) strand transfer step of the integration. A list of IN mutations for resistance to raltegravir and elvitegravir was updated in 2009 by the International AIDS Society-USA (IAS-USA) and reported in the last Stanford HIV Drug Resistance and French ANRS AC11 genotypic interpretation algorithms. Dolutegravir and MK-2048 are a new generation of INIs with the potential to inhibit HIV-1-resistant variants generated with first-generation compounds. In vitro, serial passage experiments using wild-type clinical isolates in the presence of continued dolutegravir or MK-2048 pressure led to the selection of single or combined amino acid substitutions, including L101I, T124A and S153Y/F mutations for dolutegravir,1 and two amino acid substitutions, G118R and E138K, for MK-2048.2 Most of the data about the efficacy and failure of INIs concerns HIV-1 subtype B, whereas these data are missing for the CRF02_AG subtype, which is highly prevalent in West Africa, and becoming, in recent years, more common in...
developed countries. Because variations at the nucleotide level could have the ability to drive the resistance escape pathway, there is a need for additional data for the CRF02_AG subtype.

The aim of this study was to explore all the IN mutations previously described in subtype B as involved in resistance to first-generation (raltegravir and elvitegravir) and second-generation (dolutegravir and MK-2048) INIs in 25 patients infected with the HIV-1 CRF02_AG subtype and failing a raltegravir-containing regimen.

Methods

Twenty-five HIV-1-infected patients failing to respond to 400 mg of raltegravir (two consecutive viral loads >200 copies/mL) administered twice daily, all infected with CRF02_AG subtype, were studied retrospectively. Plasma samples were collected in three clinical centres in Paris, France (Pitie-Salpe`trie`, Bichat-Claude Bernard and Saint-Antoine hospitals), and one in Rome, Italy (Department of Experimental Medicine, University of Rome ‘Tor Vergata’). Viral RNA was extracted from plasma and the complete nucleotide sequences of the IN coding region were amplified.

Phenotypic susceptibility of the viral isolates was assessed by means of a recombinant virus assay, as described previously. Briefly, viral RNA was extracted and cDNA prepared. The RT-RNAseH-IN region was amplified and sequenced as previously described. Sequences were then analysed, using CodonCode Aligner software, for the presence, in 20 positions, of previously described in vivo and/or in vitro mutations against first- and second-generation INIs: T66A/I/K, V72I, L74M, E92Q, T97A, F121Y, E138A/K, G140A/S, Y143C/H/R, S147G, Q148C/E/H/K/R, V151I, S153Y, N155H/S, E157Q, G163R and S230R for raltegravir/elvitegravir, L101I, T124A and S153F/Y for dolutegravir, and G118R and E138K for MK-2048.

Results

Twenty-five HIV-1-infected patients failing raltegravir and infected with the CRF02_AG subtype were retrospectively studied. The median plasma HIV-1 RNA level at failure was 4.5 log10 copies/mL (range 4–5.4 log10 copies/mL) and the time on raltegravir therapy until failure was, on average, 13 months (range 4–25 months). The complete nucleotide sequences of the IN coding region were obtained from patients at raltegravir failure. The sequence analysis showed the presence of several residues among the 13 previously described as specific for the CRF02_AG subtype (14R, 311, 101I, 112V/I, 124A, 125A, 134N, 135V, 136T/Q, 201I, 206S, 234I and 283G) (data not shown).

The analysis of the 20 residues implicated in resistance to raltegravir, elvitegravir, dolutegravir and MK-2048 showed that six IN residues (66, 121, 147, 151, 153 and 230) did not show any mutation in all sequences, whereas three positions (72, 101 and 124) were frequently mutated. The 101I and 124A mutations, selected in vitro by dolutegravir, were present, alone or together, in all sequences (20 sequences with 101I+124A, 3 sequences with 101I and 2 sequences with 124A). On the other hand, the 72I mutation was present in 18 sequences (72%). This mutation was previously reported as a frequent polymorphic mutation in INI-naive patients (60% in subtype CRF02_AG and 55% in subtype B), but also as a minor raltegravir resistance mutation. With the exception of these frequent mutated residues, it was observed that 7 sequences (28%) in patients p1–p7 harboured major raltegravir resistance-associated mutations (Table 1), whereas 18 sequences (72%) showed a lack of major mutations. Thus the 155H mutation, associated with raltegravir, elvitegravir, dolutegravir and MK-2048, showed a lack of major mutations. The 155H mutation, associated with 92Q, 97A or 157Q+163R, was detected in four patients, the 143R mutation, associated with 138A, was detected in one patient and the 157Q mutation alone was detected in one patient. One patient showed a mixed resistance profile with mutations 143C/H/R and 148R associated with three other mutations; 138K, 140A and 163R (Table 1). Among the 18 remaining patients, 1 patient (p8) showed an unusual association of two INI resistance-associated mutations, 74M and 118R (Table 1), the first known as a minor mutation associated with raltegravir resistance and the second one being a mutation only linked thus far to MK-2048 and S-1360 derivative resistance.

The 74M+118R profile, never described before as a resistance profile to raltegravir/elvitegravir, was further investigated. The different characteristics of the patient harbouring this profile with mutations 143C/H/R and 148R associated with three other mutations; 138K, 140A and 163R (Table 1). Among the 18 remaining patients, 1 patient (p8) showed an unusual association of two INI resistance-associated mutations, 74M and 118R (Table 1), the first known as a minor mutation associated with raltegravir resistance and the second one being a mutation only linked thus far to MK-2048 and S-1360 derivative resistance.

Table 1. IN amino acid mutations associated with resistance in vivo and/or in vitro to raltegravir, elvitegravir, dolutegravir and MK-2048 in eight patients infected with HIV-1 subtype CRF02_AG

<table>
<thead>
<tr>
<th>Mutations associated with resistance to different INIs</th>
</tr>
</thead>
<tbody>
<tr>
<td>raltegravir/elvitegravir</td>
</tr>
<tr>
<td>66</td>
</tr>
<tr>
<td>p1</td>
</tr>
<tr>
<td>p2</td>
</tr>
<tr>
<td>p3</td>
</tr>
<tr>
<td>p4</td>
</tr>
<tr>
<td>p5</td>
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<tr>
<td>p6</td>
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<tr>
<td>p7</td>
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<tr>
<td>p8</td>
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profile are detailed in Table 2. The HIV-1 load decreased from baseline to become undetectable (<40 copies/mL) in 3 months after raltegravir treatment and then returned to a value close to that of baseline at month 17 while still receiving raltegravir. The comparison of IN sequences between initiation of raltegravir treatment (day 0) and failure of raltegravir (month 17) showed the appearance of the single G118R mutation, which confers a high level of resistance to raltegravir (from 1- to 25.5-fold change at failure compared with baseline) and to elvitegravir (from 2.8- to 9.2-fold change).

Discussion

INI resistance mutations described in vivo and/or in vitro have been mostly studied for HIV-1 subtype B. Nevertheless, the selection of resistance mutations could be influenced by the naturally occurring variations between the different non-B subtypes. In this study, 25 HIV-1 IN sequences isolated from subtype CRF02_AG raltegravir-failing patients were analysed at the level of 20 amino acid positions previously described as involved in resistance to first- and second-generation INIs (raltegravir/elvitegravir and dolutegravir/MK-2048, respectively).

Our sequence analysis showed the presence of previously described major resistance mutations to raltegravir/elvitegravir associated with the 155 and 143 genetic pathways clearly established in five patients and one patient showed a 157Q mutation, previously described as linked to raltegravir failure. In spite of the small number of patients showing resistance mutations to raltegravir, we confirmed the preferential use of the 155 pathway at failure in non-B subtypes, as previously described. The fact that many of the studied patients (72%) who experienced detectable viraemia while receiving raltegravir-based antiretroviral therapy lacked previously described major resistance mutations to raltegravir/elvitegravir is surprising, but this finding is concordant with results previously reported in a large European cohort study. Further research is needed to fully elucidate this result by exploring the role of IN mutations, particularly in the non-B subtype, that are not currently recognized as major, and defining their possible implications for the use of first- as well as second-generation INIs.

In the patient p8, the unusual association of 74M and 118R mutations was observed at failure. The presence of the G118R mutation was particularly interesting for several reasons. First, several studies have shown that the G118 amino acid residue was highly conserved across many HIV-1 subtypes. Second, this mutation has never been observed before in raltegravir-failing patients and has only been selected in cell cultures after a prolonged period with MK-2048 and an S-1360 derivative. Furthermore, phenotypic analyses showed that the appearance of the single G118R mutation conferred a high level of resistance to raltegravir and elvitegravir. These results are concordant with another in vitro study showing increased fold changes to raltegravir and elvitegravir in the presence of a G118R mutation.

### Table 2. Characteristics of patient p8 and combined analysis of genotype and phenotype data of recombinant IN viruses derived from clinical isolates

<table>
<thead>
<tr>
<th>Time of raltegravir usea</th>
<th>Treatment</th>
<th>Viral load (copies/mL)</th>
<th>IN mutations relative to the HxB2 reference sequenceb</th>
</tr>
</thead>
<tbody>
<tr>
<td>D0</td>
<td>RAL, ABC, 3TC</td>
<td>787</td>
<td>E I I G I V I R V R S = A K N V T E L I D I 25.5 9.2</td>
</tr>
<tr>
<td>M1</td>
<td>RAL, ABC, 3TC, DRV</td>
<td>978</td>
<td>— S — A K N V T E I I D I 1 2.8</td>
</tr>
<tr>
<td>M2</td>
<td>RAL, ABC, 3TC</td>
<td>51</td>
<td>— S — A K N V T E I I D I 2.8</td>
</tr>
<tr>
<td>M3</td>
<td>RAL, ABC, 3TC</td>
<td>51</td>
<td>— S — A K N V T E I I D I 2.8</td>
</tr>
<tr>
<td>M5</td>
<td>RAL, ABC, 3TC</td>
<td>46</td>
<td>— S — A K N V T E I I D I 2.8</td>
</tr>
<tr>
<td>M7</td>
<td>RAL, ABC, 3TC</td>
<td>46</td>
<td>— S — A K N V T E I I D I 2.8</td>
</tr>
<tr>
<td>M9</td>
<td>RAL, ABC, 3TC</td>
<td>46</td>
<td>— S — A K N V T E I I D I 2.8</td>
</tr>
<tr>
<td>M10</td>
<td>RAL, ABC, 3TC</td>
<td>46</td>
<td>— S — A K N V T E I I D I 2.8</td>
</tr>
<tr>
<td>M12</td>
<td>RAL, ABC, 3TC</td>
<td>46</td>
<td>— S — A K N V T E I I D I 2.8</td>
</tr>
<tr>
<td>M15</td>
<td>RAL, ABC, 3TC</td>
<td>46</td>
<td>— S — A K N V T E I I D I 2.8</td>
</tr>
<tr>
<td>M17</td>
<td>RAL, ABC, 3TC</td>
<td>46</td>
<td>— S — A K N V T E I I D I 2.8</td>
</tr>
</tbody>
</table>

**Fold change values:**
- D0: 1.1
- M1: 1.2
- M2: 1.3
- M3: 1.4
- M5: 1.5
- M7: 1.6
- M9: 1.7
- M10: 1.8
- M12: 1.9
- M15: 2.0
- M17: 2.1

**RAl, raltegravir; ABC, abacavir; 3TC, lamivudine; DRV, darunavir; EVG, elvitegravir; D, day; M, month.**
mutations, resulting in an increased fold change from 16 to 37 and 9.6 to 40, respectively. In conclusion, a genetic pathway including the G118R and L74M mutations has been described in resistance to raltegravir in the context of a CRF02_AG subtype virus. This particular combination of mutations should be further evaluated for phenotypic susceptibility to second-generation INIs, i.e. dolutegravir and MK-2048.

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Transparency declarations
None to declare.

References