Primary resistance to maraviroc in a large set of R5-V3 viral sequences from HIV-1-infected patients

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\textbf{Objectives:} Evaluation of the prevalence of V3 mutational patterns associated with maraviroc resistance in R5-using variants.

\textbf{Methods:} V3 sequences were obtained from 809 plasma specimens collected from maraviroc-naive HIV-1-infected individuals on regular follow-up at Hospital Carlos III. Sequences considered to harbour R5-tropic viruses were examined for the presence of primary maraviroc resistance mutational patterns, as found in both in vitro and in vivo studies.

\textbf{Results:} A total of 498 R5-V3 sequences were identified. They belonged to recent HIV-1 seroconverters (55.6%), chronically antiretroviral-naive subjects (20.1%) and antiretroviral-experienced patients (24.3%). Most individuals (93.8%) were infected with HIV-1 subtype B. The overall prevalence of maraviroc resistance mutational patterns was low (≤5%). Likewise, specific polymorphisms 4L, 11R or 19S, recently found to be associated with lower clinical response to maraviroc, were found in 2% of tested samples. The rate of maraviroc resistance patterns did not differ significantly according to length of HIV-1 infection, antiretroviral exposure or HIV-1 subtype.

\textbf{Conclusions:} The prevalence of maraviroc resistance mutations is low in maraviroc-naive HIV-1-infected individuals.

\textbf{Keywords:} maraviroc, drug resistance, tropism, V3 loop, R5-tropic

\textbf{Introduction}

Maraviroc is the first CCR5 antagonist approved for the treatment of HIV-1 infection. Its binding to the CCR5 co-receptor leads to conformational changes in the transmembrane domain of CCR5, which inhibit the gp120–CCR5 interaction and consequently disrupt the viral entry process.\textsuperscript{1} Two main mechanisms of resistance to maraviroc have been described in patients failing on the drug. The first and most common mechanism comprises the outgrowth of pre-existing baseline X4-using variants in the presence of the drug. The second mechanism involves the selection of mutations within the HIV-1 gp120 viral envelope, which allow R5 viruses to bind the CCR5 co-receptor despite maraviroc blockade. The specific pattern of mutations that confers resistance to maraviroc in patients failing the drug and harbouring R5-tropic variants is so far unclear. Both in vitro and in vivo studies have reported different changes within gp120 that may cause maraviroc resistance. In vitro, the selection of two single mutations within the V3 loop, 21T and 28V, have been shown to confer maraviroc resistance.\textsuperscript{2} On the other hand, data from patients enrolled into the MOTIVATE trials have identified single changes (13H, 16A, 20L, G15_P16INSA, N24_I25INSI and G18DEL) and several mutational patterns (11S\textsuperscript{+}26V, 18G\textsuperscript{+}22T, 19S\textsuperscript{+}26V, 20F\textsuperscript{+}21I, 20F\textsuperscript{+}25D\textsuperscript{+}26V and G15_P16INSG\textsuperscript{+}25D), all of them located in the stem and tip of the third variable region (V3) of gp120, in patients failing maraviroc with R5-tropic variants.\textsuperscript{3} In subsequent analyses, however, the presence of these specific mutations before maraviroc treatment did not predict a higher rate of virological failure in the MOTIVATE trials.\textsuperscript{4} The only exception was for changes 4L, 11R and/or 19S, whose presence was significantly associated with a higher rate of virological failure to maraviroc, after adjusting for weighted optimized background therapy susceptibility scores.

Information about the rate of specific changes associated with maraviroc resistance in HIV-1-infected individuals naive to
Patients and methods

V3 sequences were obtained from plasma collected from HIV-1-infected individuals on regular follow-up at Hospital Carlos III, a reference HIV clinic located in Madrid, Spain. All patients had to be naive for maraviroc. The study population was split into three categories, according to length of HIV infection and antiretroviral drug exposure. The groups were as follows: recent HIV-1 seroconverters; chronically infected antiretroviral-naive subjects; and antiretroviral-experienced patients. Recent HIV-1 seroconverters were subjects with a negative test within the prior year. The V3 region was amplified and sequenced using plasma specimens, as previously described.3–5 V3 sequences were analysed using Seqscape software v2.5 (Applied Biosystems, Foster City, CA, USA), considering a nucleotide mixture when the second highest peak in the electropherogram was >25%. V3 sequences with nucleotide mixtures were expanded into all possible amino acid permutations and considered for subsequent HIV tropism interpretation. Sequences with nine or more nucleotide mixtures were excluded from the current analysis. HIV-1 tropism assessment was performed using PSSM_{HIV-1}, an optimized web-based PSSM approach that has shown very good sensitivity for the detection of X4 variants (where PSSM stands for position-specific scoring matrices).7 Specimens were considered as harbouring R5 viruses only when all permutations excluded X4-tropic strains. V3 amino acid sequences were examined and aligned with ClustalX 2.0.

The rate of distinct primary resistance changes to maraviroc was assessed considering all single mutations and patterns of mutations reported in both in vitro and in vivo studies up to date.2–4 Briefly, the following mutational patterns were examined: (i) in vitro, 21T and 28V; and (ii) in vivo, 11S+26V, 20F+25D+26V, 18G+22T, 20F+211, 19S+26V, G15_P16INSNG+25D, 13H, 16A, 20L, 4L, 11R, 19S, 22T, 26V, 20F, 11S, 25D were absent, or recent seroconverters (13.2% versus 6.1%, respectively; P = 0.03). Other less common changes, such as 16A, insertions G15_P16INSNG, G15_P16INSNA and N24_J25INSI or deletion G18DEL were not found in our study population.

Neither of the in vitro single mutations associated with maraviroc resistance (21T and 28V) was found in the study population. On the other hand, the rate of mutational patterns that have been associated with maraviroc resistance in vivo was low (<5%). The most common pattern was the double mutation 11S+26V, which was identified in 5% of R5-tropic sequences, followed by 20F+25D+26V (1.8%), 18G+22T (0.6%) and 20F+211 (0.6%). None of the patients displayed mutational patterns 19S+26V or G15_P16INSNG+25D. In eight patients, the following double mutants were identified simultaneously: 11S+26V plus 20F+25D (n = 7); or 11S+26V plus 18G+22T (n = 1). No significant differences were found in the rate of all these changes according to antiretroviral exposure or length of HIV-1 infection (Table 1).

Although the prevalence of specific mutational patterns associated with maraviroc resistance was low, some single changes included within these patterns were common. By order of frequency: 20F (86.3%); 11S (81.1%); 13H (64.3%); 25D (49.2%); 22T (22.3%); 26V (7.6%); 18G (5.8%); 20L (4.6%); 19S (0.8%); and 211 (0.6%). Of note, the frequency of these changes did not differ significantly according to antiretroviral exposure or length of HIV-1 infection, except for mutation 26V, which was more frequent in antiretroviral-experienced than in antiretroviral-naive subjects, either considering chronically infected individuals or recent seroconverters (13.2% versus 6.1%, respectively; P = 0.03). The rate of single changes newly associated with a lower rate of virological response to maraviroc in the MOTIVATE trials was as follows: 4L (1.2%); 11R (0%); and 19S (0.8%). In one subject both 4L and 19S co-existed. Again, no differences were recognized when comparing distinct groups according to length of HIV-1 infection and/or antiretroviral exposure.

Maraviroc resistance patterns were also examined in the set of 311 V3 sequences identified as X4-tropic viruses. The mutational patterns 19S+26V and G15_P16INSNG+25D were absent, as in R5-tropic viruses. No statistical differences were observed in the prevalence of resistance patterns 11S+26V, 20F+211 or 20F+25D+26V comparing X4- and R5-tropic viruses (8.7% versus 5.0%, 1.0% versus 0.6% and 1.3% versus 1.8%, respectively). Only 18G+22T was more frequently recognized in X4- than in R5-tropic viruses (3.2% versus 0.6%; P = 0.007).

Statistical analyses

All results are expressed as absolute numbers and proportions. Comparisons between groups were performed using the χ² test. All tests were two-tailed, with only P values of <0.05 considered as significant. All statistical analyses were performed using SPSS v16.0 software (SPSS Inc., Chicago, IL, USA).

Results

A total of 809 V3 sequences from 783 HIV-1-infected individuals were included in the analysis (GenBank accession numbers GU265201–GU269393). Tropism determinations performed with PSSM_{HIV-1} resulted in 311 X4- and 498 R5-tropic viruses, which were further characterized. Within R5-tropic sequences, 277 (55.6%) belonged to recent HIV-1 seroconverters, 100 (20.1%) to chronically antiretroviral-naive subjects and 121 (24.3%) to antiretroviral-experienced patients. Phylogenetic analysis of pol and env genes showed that 467 (93.8%) subjects were infected with HIV-1 subtype B, whereas 31 (6.2%) harboured non-B subtypes, distributed as follows: 9 G; 9 F1; 7 CRF02_AG; 3 A1; 1 CRF01_AE; 1 CRF12_BF; and 1 CRF14_BG.

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Table 1. Rate (%) of mutational patterns associated with maraviroc resistance in the study population

<table>
<thead>
<tr>
<th>Genotypic patterns</th>
<th>Total (n=498)</th>
<th>Seroconverters (n=277)</th>
<th>ARV naive (n=100)</th>
<th>ARV experienced (n=121)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11S+26V</td>
<td>25 (5.0)</td>
<td>11 (4.0)</td>
<td>5 (5.0)</td>
<td>9 (7.4)</td>
</tr>
<tr>
<td>20F+25D+26V</td>
<td>9 (1.8)</td>
<td>5 (1.8)</td>
<td>1 (1.0)</td>
<td>3 (2.5)</td>
</tr>
<tr>
<td>18G+22T</td>
<td>3 (0.6)</td>
<td>1 (0.4)</td>
<td>1 (1.0)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>20F+211</td>
<td>3 (0.6)</td>
<td>2 (0.7)</td>
<td>0</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>4L</td>
<td>6 (1.2)</td>
<td>3 (1.1)</td>
<td>2 (2.0)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>19S</td>
<td>4 (0.8)</td>
<td>3 (1.1)</td>
<td>1 (1.0)</td>
<td>0</td>
</tr>
</tbody>
</table>
Although other regions within gp120 may also be involved. 3 has been shown to play a pivotal role in maraviroc resistance, clades. were not examined, given the low number of cases in distinct P resistance.2 In both 2504 Given the polymorphic nature of the V3 loop, our findings are for 20F (86.3%), 11S (81.1%), 13H (64.3%) and 25D (49.2%). Changes modulating maraviroc susceptibility are mainly located at the stem and tip of the V3 region. In our study, the resistance in R5-tropic viruses were uncommon, a few single changes were frequent. This was particularly true within those patterns were frequent. This was particularly true in vivo studies have confirmed that specific changes may influence the susceptibility to maraviroc and two mutations have been shown to result in maraviroc resistance. 7 In both in vitro and in vivo studies, the V3 of gp120 has been shown to play a pivotal role in maraviroc resistance, although other regions within gp120 may also be involved. 3 Changes modulating maraviroc susceptibility are mainly located at the stem and tip of the V3 region. In our study, the presence of all these previously described changes associated with maraviroc resistance in R5-tropic viruses was only found in 5% of a heterogeneous population of 498 HIV-1-infected individuals, including subjects recently infected with HIV-1 and a subset with prior exposure to antiretroviral drugs other than maraviroc. Since maraviroc has only recently been approved for the treatment of HIV infection and its use is relatively limited, it is very unlikely that maraviroc-resistant viruses would have been recognized in our study population, supporting that the rate of resistance patterns found in this study largely reflects the high variability of the HIV envelope gene.

While mutational patterns associated with maraviroc resistance in R5-tropic viruses were uncommon, a few single changes within those patterns were frequent. This was particularly true for 20F (86.3%), 11S (81.1%), 13H (64.3%) and 25D (49.2%). Given the polymorphic nature of the V3 loop, our findings are not unexpected and are in agreement with prior reports that have examined smaller study populations. 3,10 Our findings confirm and expand the current knowledge in a larger number of HIV-1 individuals, including a subset with prior antiretroviral exposure and another of recent HIV-1 seroconverters. Moreover, the newly described specific residues 4L, 11R or 19S, which have recently been associated with an impaired virological response to maraviroc in the MOTIVATE trials, were found in <2% of tested samples. It should be acknowledged that subsequent clonal analyses of the V3 region have not confirmed the impact of 4L and 19S mutations on maraviroc resistance,11 and, therefore, the role of these changes remains unclear. Finally, antiretroviral drug exposure and length of HIV-1 infection did not seem to be associated with the selection of maraviroc-resistant mutational patterns or single changes, except for 26V, which was more common in antiretroviral-experienced patients.

In summary, the prevalence of multiple V3 mutations associated with maraviroc resistance in R5-tropic viruses is low in maraviroc-naive HIV-1-infected patients, regardless of antiretroviral treatment status and/or length of infection. Although a few single changes seem to be quite common, their impact on the genetic barrier to maraviroc when presenting alone is currently unknown. Further clinical studies are warranted to better characterize the mechanisms of maraviroc resistance in patients failing the drug with R5-tropic viruses.

Discussion
In the MOTIVATE trials, 43% of patients failing maraviroc exclusively harboured R5-tropic viruses. 8 Resistance to maraviroc in R5 variants has been associated with a reduction in the maximum percentage of inhibition. 2 The genotypic characterization of these maraviroc-resistant R5 strains has allowed the recognition of several mutation patterns as responsible for this reduced maraviroc susceptibility. In vitro studies have confirmed that specific changes may influence the susceptibility to maraviroc and two mutations have been shown to result in maraviroc resistance. 7 In both in vitro and in vivo studies, the V3 of gp120 has been shown to play a pivotal role in maraviroc resistance, although other regions within gp120 may also be involved. 3 Changes modulating maraviroc susceptibility are mainly located at the stem and tip of the V3 region. In our study, the presence of all these previously described changes associated with maraviroc resistance in R5-tropic viruses was only found in 5% of a heterogeneous population of 498 HIV-1-infected individuals, including subjects recently infected with HIV-1 and a subset with prior exposure to antiretroviral drugs other than maraviroc. Since maraviroc has only recently been approved for the treatment of HIV infection and its use is relatively limited, it is very unlikely that maraviroc-resistant viruses would have been recognized in our study population, supporting that the rate of resistance patterns found in this study largely reflects the high variability of the HIV envelope gene.

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References

Transparency declarations
None to declare.