Prevalence of etravirine-associated mutations in clinical samples with resistance to nevirapine and efavirenz

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Objectives: To evaluate the expected activity of etravirine in clinical samples, according to mutational patterns associated with decreased virological response (VR).

Methods: We identified 1586 routine clinical samples with resistance-associated mutations (RAMs) to nevirapine and efavirenz (K103N 60%, Y181C 37%, G190A 27%, V108I 13%). Concerning in vitro identified etravirine mutations, samples with F227C, Y181I, M230L or L100I plus K103N plus Y181C were considered highly resistant. Samples with two RAMs plus Y181C or V179D or K101E or Y188L were considered intermediate. The prevalence of 13 RAMs recently associated with decreased VR to etravirine in the DUET clinical trials was also investigated.

Results: Most samples (69%) harboured more than one IAS-USA RAM to first-generation non-nucleoside reverse transcriptase inhibitors (NNRTIs): 42% harboured two RAMs, 21% three RAMs and 6% four or more RAMs. The prevalence of 13 specific etravirine RAMs was V179F 0.12%, G190S 3.9%, Y181V 0.1%, V106I 2.6%, V179D 1.6%, K101P 2.0%, K101E 10.1%, Y181C 36.9%, A98G 5.9%, V90I 6.9%, Y181I 3.6%, G190A 27% and L100I 9.1%. The five RAMs with the most impact on VR (V179F/D, G190S, Y181V and V106I) occurred less often. Overall, 8.2% of the samples had three or more etravirine RAMs and only 1.1% had four or more. In addition, patterns of RAMs previously associated with intermediate etravirine resistance were present in 26.2% of the samples, whereas 4.85% displayed patterns of high-degree resistance.

Conclusions: For RAMs associated with decreased VR, etravirine resistance in routine clinical samples was lower than previously reported. High-degree resistance was uncommon, even in patients with resistance to first-generation NNRTIs, whereas low-to-intermediate etravirine resistance was more common.

Keywords: non-nucleoside reverse transcriptase inhibitors, TMC125, resistance-associated mutations

Introduction

The efficacy of first-generation non-nucleoside reverse transcriptase inhibitors (NNRTIs) is limited by their low genetic barrier to resistance, resulting from the relatively easy selection of single mutations that confer nearly complete cross-resistance. Resistance to first-generation NNRTIs among patients with treatment failure is widespread, given that they have been extensively used in clinical practice.1,2 Etravirine is a new NNRTI with expanded activity against HIV-1 resistant to current NNRTIs and has demonstrated its efficacy and favourable safety profile in double-blind, placebo-controlled trials with treatment-experienced patients (DUET studies).3,4

The development of resistance to etravirine is complex and requires the coexistence of several specific resistance-associated mutations (RAMs). In vitro studies identified mutational patterns associated with increased resistance. Using pooled data from DUET studies, only 13 of those RAMs at eight positions were associated with decreased virological response (VR) at week 24.5

We assessed the expected activity of etravirine in samples with resistance to first-generation NNRTIs by searching for mutational patterns described both in vitro during etravirine development and those validated in vivo in DUET trials.

Methods

In a systematic database search of 4981 samples from patients, which had been submitted to our laboratory for routine clinical resistance testing between 1998 and 2006, we identified 1586
different patients with documented RAMs conferring resistance to nevirapine or efavirenz. The search included any RAM conferring resistance to the first-generation NNRTIs nevirapine or efavirenz present in the IAS-USA Drug Resistance Mutation List, namely L100I, K103N, V106A/M, V108I, Y181C/I, Y188C/H/I/L, G190S/A or P225H.6

The IrsiCaixa Foundation based in Barcelona, Spain, is a reference retrovirology laboratory receiving samples for routine genotyping. HIV-1 DNA coding for amino acids between positions 37 and 247 of reverse transcriptase (RT) is routinely sequenced and genotyped using the FDA-approved TRUGENE™ HIV-1 genotyping kit (Siemens).

Sets of mutations evaluated

Mutational patterns conferring resistance to etravirine were identified by comprehensively searching peer-reviewed journals and presentations at medical conferences. The survey gathered mutations identified in vitro and in vivo. In vitro studies, including standardized sequential passage experiments at low and high multiplicity of infection as well as site-directed mutant analysis, undertaken during drug development identified mutations specifically selected by etravirine. High-level resistance was associated with the presence of either F227C, Y181I or M230L mutations alone or L100I plus K103N plus Y181C mutations, or the presence of two or more first-generation NNRTI RAMs associated with K101P or V179D/E/F/I or Y181I/V or G190S.7,8 Two first-generation NNRTI RAMs plus Y181I/V or V179D/E/F or K101E/P or Y188L were considered as conferring intermediate resistance and were associated with fold change (FC) increases in EC₅₀ values of 4–10.7,9 L100I plus K103N has also been identified as conferring low intermediate resistance to etravirine.7

Vingerhoets et al.5 found a correlation between 13 RAMs and clinical response (decreased VR) to etravirine in the phase III DUET trials. They identified the following mutations: V90I, A98G, L100I, K101E/P, V106I, V179D/F, Y181C/I/V and G190A/S. The investigators analysed only 26 of 44 potential RT RAMs present at study entry in five or more participants. VR was defined as HIV-1 RNA <50 copies/mL at 24 weeks, and an arbitrary line was drawn at a 25% response reduction. Patterns of RAMs were, therefore, only judged to be associated with a significant loss of activity if <75% of the patients achieved <50 copies/mL. No single RAM had a significant impact on VR by itself. However, all etravirine RAMs occurred mainly with other NNRTI RAMs. In the multivariate analysis, patients with one or two etravirine RAMs displayed a 19% decrease in VR, whereas the VR dropped to below 75% in patients with three or more RAMs. Therefore, the presence of one to two of these etravirine RAMs was considered as partial or low-level resistance and the presence of three or more RAMs as high-level resistance.

We analysed descriptively the etravirine RAMs, calculating means and percentages.

Results

Of the 4981 samples submitted for routine clinical resistance testing, 1586 (31.8%) had mutations conferring resistance to nevirapine and efavirenz. Of these, 97.2% were subtype B. Among these non-B samples, subtypes were 25% CRF02_AG, 16% F1, 13.6% C, 11.3% CRF12_BF, 2.3% A2, 2.3% D; in 29.5% the subtype could not be assigned. The most frequent mutations were K103N (59.7%) and Y181C (37%). The frequencies of all mutations are depicted in Figure 1. In total, 31%

Figure 1. Prevalence of first-generation NNRTI and specific etravirine-related mutations with clinical impact on etravirine response in routine clinical samples with resistance to nevirapine or efavirenz (1998–2006).
Etravirine mutations in clinical samples

Table 1. Frequency of patterns of combinations of mutations conferring resistance to etravirine

<table>
<thead>
<tr>
<th>RAMs</th>
<th>In vitro combinations</th>
<th>Clinically validated (DUET studies)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>high-degree resistance [n (%)]</td>
<td>intermediate-degree resistance [n (%)]</td>
</tr>
<tr>
<td>K101P + ≥2 mut</td>
<td>32 (2.01)</td>
<td>—</td>
</tr>
<tr>
<td>V179E + ≥2 mut</td>
<td>5 (0.31)</td>
<td>—</td>
</tr>
<tr>
<td>V179F + ≥2 mut</td>
<td>5 (0.31)</td>
<td>—</td>
</tr>
<tr>
<td>Y181V + ≥2 mut</td>
<td>2 (0.12)</td>
<td>—</td>
</tr>
<tr>
<td>Y181I + ≥2 mut</td>
<td>41 (2.58)</td>
<td>—</td>
</tr>
<tr>
<td>G190S + ≥2 mut</td>
<td>19 (1.2)</td>
<td>—</td>
</tr>
<tr>
<td>M230L + ≥2 mut</td>
<td>7 (0.4)</td>
<td>—</td>
</tr>
<tr>
<td>F227C alone</td>
<td>2 (0.13)</td>
<td>—</td>
</tr>
<tr>
<td>Y181I alone</td>
<td>57 (3.6)</td>
<td>—</td>
</tr>
<tr>
<td>Y181C + ≥2 mut</td>
<td>—</td>
<td>251 (15.82)</td>
</tr>
<tr>
<td>K101E + ≥2 mut</td>
<td>—</td>
<td>105 (6.62)</td>
</tr>
<tr>
<td>L100I + ≥2 mut</td>
<td>—</td>
<td>104 (6.55)</td>
</tr>
<tr>
<td>Y188L + ≥2 mut</td>
<td>—</td>
<td>28 (1.76)</td>
</tr>
<tr>
<td>V179D + ≥2 mut</td>
<td>—</td>
<td>18 (1.13)</td>
</tr>
<tr>
<td>G190A + ≥2 mut</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>V106I + ≥2 mut</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A98G + ≥2 mut</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>V90I + ≥2 mut</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>L100I + ≥2 mut</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>≥3 Etravirine mut</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*mut, mutation(s); n, number of patients; RAMs, resistance-associated mutation(s); VR, virological response.*

The most frequent RAMs related to any decreased etravirine activity were Y181C (36.9%), G190A (27%), K101E (10.1%), L100I (9.1%), V90I (6.9%), Y188L (6.1%), V179I (6%), A98G (5.9%), G190S (3.9%), Y181I (3.6%), V106I (2.6%) and K101P (2%). The prevalence of the remaining RAMs was <1% (Figure 1).

Analysis of RAMs identified during etravirine drug development (in vitro)

The general prevalence of any mutation or combination of mutations reported to confer high or intermediate degrees of resistance was 31%.

Mutational patterns reported to confer high-degree resistance were found in 4.85% of the samples (77/1586). The prevalence of single RAMs associated with high-degree etravirine resistance was 0.1% for F227C, 3.6% for Y181I and 0.8% for M230L. The most frequent combinations were Y181I plus two or more mutations (2.58%), K101P plus two or more mutations (2.01%) and K103N plus L100I plus Y181C (0.36%). The remaining mutational patterns appeared in <1% (Table 1). The combination of V179D/F or Y181I or G190S or M230L plus four mutations, reported to confer even higher resistance to etravirine, was extremely rare (0.75% overall).

RAM patterns associated with intermediate resistance were identified in 26.3% of the samples (417/1586), the most frequent being the Y181C plus two or more mutations (15.82%), K101E plus two or more mutations (6.62%), L100I plus K103N (6.55%), Y188L plus two mutations (1.76%) and V179D plus two mutations (1.13%) (Table 1).

Analysis of clinically validated etravirine RAMs in the DUET studies

The most frequent mutations validated to confer resistance in the DUET studies were Y181C (36.9%), G190A (27%), K101E (10.1%) and L100I (9.1%), but the five RAMs with the highest impact on VR (V179F/D, G190S, Y181V and V106I) were found less frequently in our clinical samples (Figure 1). With regard to combinations, 8.1% of the samples had three or more specific etravirine RAMs, Y181C plus two or more mutations (6.5%) and K101E plus two or more mutations (4.98%) being the most frequently identified. Only 1.13% of the samples had four or more etravirine-associated RAMs. The remaining combinations are depicted in Table 1.

Combinations with four or more NNRTI RAMs were found in 90 (6%) samples, and 54 (60%) of them shared Y181C. Likewise, 18 (1.14%) samples had four or more etravirine-specific RAMs and 67% (n = 12) contained the Y181C mutation.

Discussion

According to our analysis, high-level etravirine resistance was uncommon in HIV-1 infected patients with resistance to first-generation NNRTIs in routine clinical practice, regardless of

(n = 491) of the samples had only one NNRTI RAM, 42% (n = 670) had two, 21% (n = 333) had three and 6% (n = 90) of the samples had four or more mutations.

The most frequent RAMs related to any decreased etravirine activity were Y181C (36.9%), G190A (27%), K101E (10.1%), L100I (9.1%), V90I (6.9%), Y188L (6.1%), V179I (6%), A98G (5.9%), G190S (3.9%), Y181I (3.6%), V106I (2.6%) and K101P (2%). The prevalence of the remaining RAMs was <1% (Figure 1).
whether mutation patterns reported during drug development or those clinically validated in the DUET trials are considered. Samples with low-to-intermediate resistance are much more prevalent, although when only RAMs associated with decreased VR in the DUET trials are considered, the prevalence of etravirine resistance is lower than previously reported during drug development.

After K103N, Y181C was the most frequent NNRTI-related mutation (37%), in line with previous reports, perhaps related to the wider use of nevirapine in Spain. Neither individual mutation has been associated with etravirine resistance by itself, although most highly resistant clones in vitro contain Y181C. In addition, Y181C in combination with two or more additional mutations has been associated in vitro and in vivo with increased etravirine resistance. We found a high prevalence of Y181C associated with two additional NNRTI mutations, and patients with four or more NNRTI mutations or etravirine-specific RAMs usually harboured Y181C. When such mutations were present, etravirine would not be a preferred drug to include in salvage regimens.

In our analysis, the frequency of L100I was 9%, which is slightly higher than other reports but similar to another one in which the prevalence was calculated from a database containing 7144 clinical samples. In vitro, intermediate etravirine resistance was reported for L100I plus K103N, for which a fold change (FC) of 11 was reported, although the FC for each individual mutation was 2.1 and 0.5, respectively. In our analysis, L100I plus K103N constituted the most frequent combination associated with etravirine resistance. However, in DUET trials, L100I was associated with the smallest decrease in VR among the identified set of mutations. It rarely appears alone, and it is associated with a median of two NNRTI RAMs. A more detailed analysis of its impact with every specific RAM is required because it is very common in patients with prior failure to first-generation NNRTIs.

V179I, another mutation reported during etravirine development and a common polymorphism in HIV subtype A, was present in 5.9% of the patients. However, this mutation has not been validated in DUET studies, even though insertions F and D at position 179 were included, and V179E is under evaluation. Although a recent study has also associated V179I with etravirine resistance, its role remains unclear and should be studied further.

Other mutations, both clinically validated (K101P, Y181I/V, G190S and V179F) and reported in vitro (V179E, G190E, F227L and M230L), had frequencies below 2% in our study (except Y181I), in agreement with previous reports. The prevalence of combinations of V179E/D/F or Y181I or G190S or M230L plus four mutations, reported to confer even higher resistance to etravirine, was extremely rare (0.75% overall).

DUET trials showed that an increasing number of baseline etravirine RAM was associated with a steady decrease in VR, with the greatest impact in patients with three etravirine RAMs. However, the specific relevance of each mutation is still to be determined in the unweighted score. In our analysis, 27% of the samples had three or more of these etravirine mutations. This is slightly higher than reported in DUET trials, perhaps because of more widespread prior use of nevirapine in Spain driving Y181C selection. In contrast, rates of V106I, G190S, V179F and Y181V, which had the most pronounced effect on etravirine VR, were lower in our study.

Our analysis is limited in that etravirine is a novel drug with modest clinical experience with unweighted mutation scores, pending further fine-tuning in the future. Both in vitro and clinically validated scores have advantages and drawbacks, and in vitro experiments do not always correspond with in vivo results, particularly in salvage trials with complex antiretroviral regimens. Previous studies had based the analysis of etravirine resistance on a phenotypic FC >10, but this is an arbitrary unvalidated threshold. The prevalence of additional mutations such as T386A and Y318A could not be assessed in our study, because mutations beyond the 247 position were not routinely amplified.

This analysis shows that the prevalence of high-level resistance to etravirine is low in routine clinical practice, and the drug should retain activity in most patients with resistance to first-generation NNRTIs. Nevertheless, the prevalence of mutations or their combinations associated with low-to-intermediate etravirine resistance is quite common. Our findings, therefore, support the recommendation of early withdrawal of first-generation NNRTIs from non-suppressive antiretroviral regimens to avoid the accumulation of further mutations that would jeopardize future etravirine activity.

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Transparency declarations

None to declare.

References


912
Etravirine mutations in clinical samples


