POSTER 868
CROI 2008 BOSTON
15TH CONFERENCE on Retroviruses and Opportunistic Infections

PREVALENCE OF MUTATIONS WITH IMPACT ON VIROLOGICAL RESPONSE TO ETRAVIRINE IN ROUTINE CLINICAL SAMPLES

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ABSTRACT

BACKGROUND

To evaluate the prevalence of mutations (RAMs) associated with diminished virological response to Etravirine (ETV) in the DUET trials in routine clinical samples with resistance to first generation NNRTI.

METHODS

We identified 1586 out of 4981 (31.8%) samples submitted for routine clinical resistance testing from 1998-2006 with resistance to first generation NNRTI (one RAM in the reverse transcriptase gene associated with resistance to NNRTI in the IAS-USA list).

RESULTS

Most of the samples (68.9%) harboured >1 IAS-USA RAMs to first generation NNRTIs: 670 (42.2%) harboured 2 RAMs, 333 (21%) 3 RAMs, and 90 (5.7%) ≥4 RAMs. The prevalence of specific ETV RAMs associated with decreased virological response (ranked in decreasing order by their impact on virological response) was: V179F 0.12%, G190S 1.0%, Y181V 0.1%, V106I 0.75%, V179D 0.75%, K101P 1.38%, K101E 1.89%, Y181C 4%, A98G 5.9%, V90I 6.9%, Y181I 3.6%, G190A 3.65%, L100I 9.1%. The top five mutations with higher impact on the virologic response (V179F, G190S, Y181V, V106I, V179D) showed the lower occurrence rates. The overall prevalence of samples with 3 ETV-associated RAMs was 8.2%, whereas only 1.14% of samples had ≥4 ETV-associated RAMs. Patterns of mutations previously associated with intermediate ETV resistance in vitro or during ETV development (Y181C + 2 RAMs, V179D + 2 RAMs, K101E + 2 RAMs, Y188L + 2 RAMs, L100I + K103N) were detected in 417 (26.2%) samples, while 77 (4.85%) further samples (31% in total) displayed patterns associated with high degree resistance in vitro (F227C, Y181I/V + 2 RAMs, M230L + 2 RAMs, G190S + 2 RAMs, V179E/D/F + 4 RAMs, G190S + 3 RAMs, K101P + 2 RAMs).

Resistance to ETV in routinely collected clinical specimens considering only RAMs associated with decreased virological response is lower than previously reported. High-degree resistance to ETV is rare, even in patients with resistance to first generation NNRTIs. However, low-to-intermediate ETV resistance is rather more common. These results further support the recommendation of early withdrawal of first generation NNRTI from non-suppressive antiretroviral regimens in order to avoid the accumulation of further mutations that would endanger ETR activity.

INTRODUCTION

Resistance to NNRTI among HIV-1 patients highly experienced to antiretroviral therapy is widespread and the prevalence of transmitted NNRTI mutations is also increasing in some populations1,2. Therefore, there is a growing need for the development of new NNRTI with better resistance profiles and with activity against NNRTI resistant HIV-1 strains.

Etravirine (ETV), formerly TMC125, is a new NNRTI with expanded activity against HIV-1 with resistance against current NNRTI, and has demonstrated its efficacy and favourable safety profile in double-blind, placebo controlled, trials with treatment experienced patients (DUET studies).3,4

In vitro studies undertaken during the drug development, identified mutational patterns associated with increased ETV resistance. In addition, using pooled data from the DUET studies only 13 of those resistance-associated mutations (RAMs) at 8 positions were associated with ETV decreased virological response (VR) at week 24.5

We assessed the foretold activity of ETV in samples with resistance to first generation NNRTIs considering both mutational patterns: those described in vitro and during ETV development 6-8, and those validated in vivo in the DUET trials.9

METHODS

We searched retrospectively at the Irsicaixa Foundation (Reference HIV-1 Resistance Lab) all samples submitted for routine clinical resistance testing from 1998-2006, and identified 1586 out of 4981 samples of patients with documented RAMs to nevirapine or efavirenz (delavirdine was not included). The search included any NNRTI RAM present in the IAS-USA Drug Resistance Mutation List of L100I, K103N, V106A/M, V108I, Y181C/I, Y188C/H/L, G190S/A, or P225H. 10 Statistical analysis was descriptive with calculation of means and percentages. In patients with more of one sample sequentially genotyped we selected only the sample with more ETV RAMs.

Mutational patterns conferring resistance to ETV were identified after a comprehensive bibliographic search including peer-reviewed journals and presentations at medical conferences. The survey gathered mutations identified in vitro and in vivo. 2,3,5-7 In vitro studies undertaken during ETV drug development identified mutations specifically selected by ETV. These studies included standardized sequential passage experiments at low and high multiplicity of infection, and with site-directed mutants as well. The prevalence of ETV RAMs associated with decreased VR and clinically validated in the DUET studies was also investigated.

RESULTS

Most samples (69%) harboured >1 IAS-USA RAMs to first generation NNRTIs, 42% harboured 2 RAMs, 21% 3 RAMs, and 6% ≥4 RAMs. The more frequent mutations were K103N (59.7%) and Y181C (37%). The whole frequencies of mutations are depicted in Figure 1.

1. Analysis of RAMs reported during in vitro ETV drug development.

The general prevalence of any previously reported mutation or combination of mutations isolated during ETV drug development in vitro and conferring higher or intermediate degree of resistance to ETV was 31%.

Of these, 26.2% (417/1586) were intermediate resistant (Y181C + 2 RAMs, V179D + 2 RAMs, K101E + 2 RAMs, Y188L + 2 RAMs, L100I + K103N), while 4.85% (77/1586) displayed patterns associated with high-degree resistance (F227C alone, Y181I/V + 2 RAMs, M230L + 2 RAMs, G190S + 2 RAMs, Y181I/V + 2 RAMs, V179E/D/F + 4 RAMs, G190S + 3 RAMs, K101P + 2 RAMs).

The rates of all combinations of RAMs related to ETV resistance reported during ETV development are shown in Table 1.

2. Analysis of ETV RAMs clinically validated in DUET studies.

The absolute frequency of single ETV-associated RAMs are depicted in figure 1. The most frequent were Y181C, G190A and K101E. The prevalence of ETV RAMs associated with decreased virological response in the DUET studies analysis (ranked in decreasing order by their impact on virological response in the studies) was: V179F 0.1%, Y181V 0.1%, V179D 1.6%, V106I 2.6%, G190S 3.9%, K101P 2.0%, K101E 10.1%, Y181C 36.9%, A98G 5.9%, V90I 6.9%, Y181I 3.6%, G190A 27%, L100I 9.1%.

The top five mutations with higher impact on the virologic response (V179F, G190S, Y181V, V106I, V179D in decreasing order of impact on VR) actually showed the lower occurrence rates in our clinical samples.

Y181C is by far the most frequent RAM with clinical impact on ETV response in our analysis of samples with resistance to first generation NNRTIs. The prevalence of Y181C associated with ≥ 2 additional NNRTIs mutations is considerable (15.82%), and 60% of those samples with 4 or more mutations include Y181C.

The overall prevalence of samples with 3 ETV-associated RAMs was 8.2%, whereas only 1.14% of samples had ≥ 4 ETV-associated RAMs.

Figure 1. Prevalence in routine samples with resistance to NVP or EFV (1998-2006) of classical NNRTI mutations, and specific ETV-related mutations with clinical impact on ETV response.

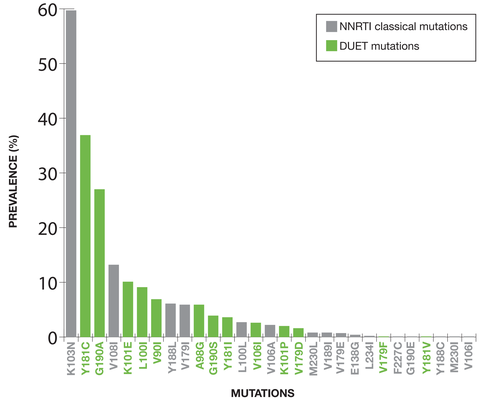

Table 1. Frequency of ETV related combinations mutations

RAMs	IN VITRO COMBINATIONS		CLINICALLY VALIDATES (DUET STUDIES)*
	High degree Resistance N (%)	Intermediate Resistance N (%)	Resistance and Lost of VR N(%)
K101P + ≥ 2muts	32 (2.01)	-	10 (0.63)
V179E + ≥ 2muts	5 (0.31)	-	-
V179F + ≥ 2muts	5 (0.31)	-	4 (0.25)
Y181V + ≥ 2muts	2 (0.12)	-	0
Y181I + ≥ 2muts	41 (2.58)	-	15 (0.94)
G190S + ≥2muts	19 (1.2)	-	14 (0.88)
M230L + ≥2muts	7 (0.4)	-	-
F227C alone	2 (0.13)	-	-
Y181I alone	57 (3.6)	-	-
Y181C + ≥2muts	-	251 (15.82)	104 (6.55)
K101E + ≥2muts	-	105 (6.62)	79 (4.98)
L100I + K103N	-	104 (6.55)	-
Y188L + ≥2muts	-	28 (1.76)	-
V179D + ≥2muts	-	18(1.13)	8 (0.50)
G190A+ ≥3mut	-	-	15 (0.94)
V106I + ≥2mut	-	-	11 (0.69)
A98G + ≥2mut	-	-	30 (1.89)
V90I + ≥2 mut	-	-	26 (1.63)
L100I + ≥2mut	-	-	8 (0.50)
≥3 ETV mut	-	-	130 (8.1)

CONCLUSIONS

ETV will retain activity in most patients with resistance to first generation NNRTI.

Complete resistance to ETV will be very rare, but low-to-intermediate resistance will be quite common.

The frequency of samples with 3 or more NNRTI mutations is common in patients failing to first generation NNRTI in the clinical practice, but the prevalence of ETV specific mutations (V179F, G190S, Y181V, V106I and V179D) with the most pronounced effect in ETV activity is low.

The Y181C is present in most cases harbouring 3 or more NNRTI-related mutations.

These results emphasize the recommendation of early withdrawal of first generation NNRTIs from non-suppressive antiretroviral regimens in order to avoid the accumulation of further mutations that would increase the degree of resistance to ETV. In addition, the specific impact in ETV response of some ETV RAMs when they occur in the presence of other NNRTI RAMs must be further investigated.

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Fundació irsiCaixa

Germans Trias i Pujol Hospital