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An update of the list of NNRTI mutations associated with decreased virologic response to etravirine (ETR): multivariate analyses on the pooled DUET-1 and DUET-2 clinical trial data

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Abstract

Background

Etravirine (ETR; TMC125) is a next-generation NNRTI with activity against NNRTI-resistant HIV-1 and a high genetic barrier to the development of resistance. Analyses of the pooled DUET-1 and DUET-2 Phase III clinical trials identified 13 ETR resistance-associated mutations (RAMs) (V90I, A98G, L100I, K101E/P, V106I, V179D/F, Y181C/I/V, and G190A/S). The presence of three or more of these RAMs was associated with decreased virologic response to ETR. In this study, additional statistical approaches were used to refine this list and improve the genotype/phenotype correlation.

Methods

Effect of baseline resistance on virologic response (<50 copies/mL) to ETR at Week 24 was studied in patients not using enfuvirtide (ENF) *de novo* and excluding those who discontinued for other reasons than virologic failure (n=406). Multivariate analyses included logistic regression controlling for baseline viral load, darunavir (DRV) fold change in 50% effective concentration (FC) and NRTI sensitivity. Mutations were identified based on the association with decreased virologic response and/or increased ETR FC. Mutations in the reverse transcriptase (RT; amino-acids 1–400) were included in the final analysis if present in ≥5 patients.

Results

The analyses confirmed the impact on response of the 13 ETR RAMs identified previously and also identified K101H, E138A and V179T as associated with a decreased virologic response and/or increased ETR FC. The V179F/T, Y181V, and G190S mutations were associated with the lowest virologic response, but were present in <5% of patients at baseline. Virologic response decreased in subgroups with increasing numbers of baseline ETR RAMs (77%, 61%, 56%, 38% for 0, 1, 2, ≥3 RAMs, respectively). Relative weighting of the 16 ETR RAMs improved the correlation between baseline ETR FC and the number of ETR RAMs.

Conclusions

A comprehensive analysis of baseline resistance data from DUET-1 and DUET-2 identified three additional mutations resulting in a list of 16 ETR RAMs (V90I, A98G, L100I, K101E/H/P, V106I, E138A, V179D/F/T, Y181C/I/V, and G190A/S). Weighting these mutations improved the correlation between genotypic and phenotypic resistance interpretations. Decreased virologic response was a function of the number of baseline ETR RAMs with the largest impact observed in the subgroup of patients with three or more RAMs.

Please note some of the data in the abstract have been updated in the poster

Background

- ETR is a next-generation NNRTI, with activity against NNRTI-resistant HIV-1 and a high genetic barrier to the development of resistance
- Previous analyses of the pooled DUET-1 and DUET-2 Phase III clinical trials identified 13 ETR RAMs (i.e. 2007 list of ETR RAMs: V90I, A98G, L100I, K101E/P, V106I, V179D/F, Y181C/I/V, and G190A/S)[1]
- The presence of three or more of these RAMs was associated with decreased virologic response to ETR[1]

[1]Vingerhoets J, et al. IHDRW 2007 Abstract 32

Objectives

- Further explore the baseline mutations associated with decreased virologic response to ETR, in the pooled DUET-1 and DUET-2 trials
- Develop a weighted ETR mutation score
  - to improve the agreement with the ETR phenotypic susceptibility interpretations (see Peeters M, et al. IHDRW 2008, Abstract 121)
  - to provide better guidance in the interpretation of ETR resistance

Methods

Selection of mutations

- The list of 44 NNRTI RAMs was expanded to 57 mutations by addition of all mutations observed at NNRTI resistance amino acid positions (Table 1)
- The main selection criterion was the effect of baseline mutations on virologic response (<50 copies/mL) to ETR at Week 24 – studied in patients not using ENF *de novo* and excluding those who discontinued for other reasons than virologic failure (n=406) (Table 1 and Figure 1)
- Other criteria to select mutations were based on their association with increased ETR FC (Tables 1 and 2)

Methods (cont'd)

Weighting of mutations

- Relative weights were determined using random forest and linear model techniques using matched genotype/phenotype data from DUET (n=406) and from a panel of NNRTI-resistant HIV-1 recombinant clinical isolates (n=4,248) (Figures 2 and 3)
- The results of these analyses are shown in Tables 2 and 3 and Figures 4, 5 and 6
- A confirmatory multivariate analysis included logistic regression controlling for baseline viral load (VL), DRV FC and NRTI sensitivity

Table 1. Overview of the NNRTI mutations

- The list of 44 NNRTI RAMs was expanded to 57 mutations by addition of all mutations at NNRTI resistance amino acid positions
- The frequency and virologic response in patients with the mutations at baseline is shown (if n≥5), along with the ETR FC in a site-directed mutant (SDM)
- M230L was added based on a strong effect on the FC, although n=4
- L100I was included previously, although the response rate was above the threshold (see Figure 1)

ETR RAMs 2008
Response rate below threshold (<51.9%)
ETR FC >3.0

Mutation	No. of patients with mutation at baseline	No. of patients with <50 cps/mL at Week 24	Response rate (<50 cps/mL) in pooled DUET (%)	ETR FC in single SDM
V90I	22	11	50.0	1.5
A98G	59	29	49.2	2.5
A98S	38	26	68.4	0.4
L100I	34	18	52.9	1.8
K101E	53	24	45.3	1.7
K101H	26	11	42.3	1.3
K101P	9	4	44.4	6.2
K101Q	35	20	57.1	0.4
K101R	5	3	60.0	0.7
K103N	118	82	69.5	0.7
K103R	9	2	22.2	0.8
K103S	16	12	75.0	0.9
V106A	24	9	37.5	NA
V106I	66	42	63.6	0.5
E138G	12	6	50.0	2.0
E138Q	10	8	80.0	5.1
V179D	5	2	40.0	2.6
V179F	7	1	14.3	0.1
V179I	97	60	61.9	0.8
V179T	8	3	37.5	0.8
Y181C	110	50	45.5	3.9
Y181I	8	4	50.0	12.5
Y181V	6	2	33.3	17.4
Y188L	32	24	75.0	0.9
V189I	24	13	54.2	0.8
G190A	115	58	50.4	0.8
G190S	14	3	21.4	0.2
H221Y	35	23	65.7	2.5
P225H	5	3	60.0	1
F227L	20	13	65.0	0.4
M230L	4	2	50.0	3.4
K238T	10	9	90.0	2.4
Y318F	13	10	76.9	1.4
N348I	60	35	58.3	NA

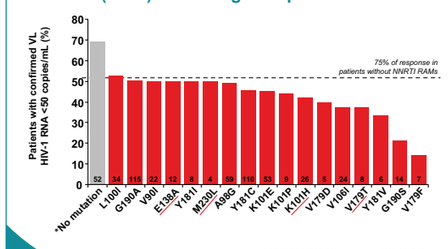
The following mutations were present in <5 patients at baseline: K101N, K103H, K103T, V106A, V106M, E138G, E138K, V179A, V179E, V179G, Y181F, Y188C, Y188F, Y188H, G190C, G190E, G190Q, G190R, F227C, M230, M230L, P236L, K238N and N348T

Table 2. Weight factors – 2008 ETR RAMs

Mutation	Prevalence (%) in the panel of 4,248 HIV-1 clinical isolates	ETR FC in the subset of HIV-1 clinical isolates with 1 ETR RAM (n=1,619), regardless of the presence of other NRTI or NNRTI RAMs* Median	Q1–Q3	n	ETR FC in a single site directed mutant	Effect on FC in linear model	Weight factor
Y181I	1.5	42.0	23.2–129.7	34	12.5	High	3
Y181V	0.9	10.4	3.9–60.6	28	17.4	High	3
K101P	2.6	22.3	5.6–42.9	65	6.2	High	2.5
L100I	8.4	6.7	2.7–17	264	1.8	Medium	2.5
Y181C	32.0	4.4	2.1–11.6	552	3.9	Medium	2.5
M230L	1.1	4.3	2.7–10.5	20	3.4	High	2.5
E138A	2.5	2.9	1.4–10.6	44	2.0	Medium	1.5
V106I	4.4	2.6	1.4–5.2	63	NA	Low	1.5
G190S	3.7	0.8	0.6–1.7	32	0.2	Low	1.5
V179F§	0.7	–	–	0	0.1	Medium	1.5
V90I	6.8	2.0	0.8–3.6	97	1.5	Low	1
V179D	2.1	1.7	1.0–4.7	33	2.6	Low	1
K101E	9.9	1.5	0.8–2.5	24	1.7	Low	1
K101H	2.2	1.1	0.6–2.8	8	1.3	Low	1
A98G	9.5	1.0	0.5–1.9	127	2.5	Low	1
V179T	0.6	0.9	0.7–1.2	2	0.8	Low	1
G190A	23.3	0.8	0.5–1.5	226	0.8	Low	1

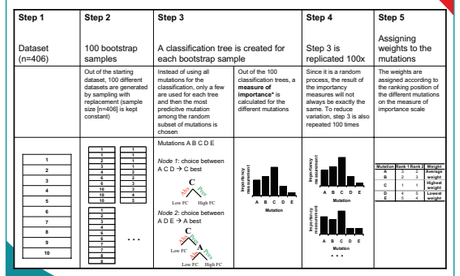
*Median (Q1–Q3) FC for all isolates was 3.0 (1.1–9.3)
§V179F was never present as single ETR RAM (always with Y181C)

Figure 1. Effect of the ETR RAMs 2008 (n=17) on virologic response



*No detectable baseline NNRTI RAM from the list of 44; Mutations in the ETR RAM list of 2008 but not 2007 are underlined

Figure 2. Random forest analysis



*The measurement of importance is the total decrease in node impurities (incorrectness) from splitting on that specific mutation, averaged over all trees

Figure 3. Linear model analysis

Dataset = recombinant HIV-1 clinical isolates

For every mutation (mut_i):
- 0 when mutation is absent
- 1 when mutation is present (not in mixture)
- 1/2, 1/3 for mixtures of 2 or 3 amino acids, respectively

Model 1

$$\log FC = \beta_0 + \beta_1 mut_1 + \beta_2 mut_2 + \cdots + \beta_n mut_n$$

- All single mutations (with n≥20)
- Stepwise regression: mutations are added to and removed from the model based on the following parameters
  - p value threshold to enter the model = 0.5
  - p value threshold to stay in the model = 0.0001

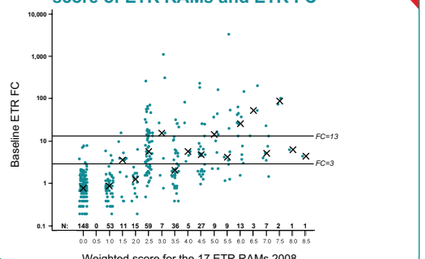
→ in each forward step, the most predictive mutation is selected, and in the subsequent backward step, the more stringent p value allows elimination of the least predictive mutations

Model 2

$$\log FC = \beta_0 + \beta_1 mut_1 + \beta_2 mut_2 + \cdots + \beta_n mut_n + \beta_{12} mut_1 * mut_2 + \ldots + \beta_{n2} mut_2 * mut_n$$

- Performed using all mutations from Model 1 in addition to any pairwise combinations with those mutations
- Interactions are taken into account if the combination occurs in at least 20 samples and if each mutation of the pair was present in at least 20 samples separately.
- **Stepwise regression** – p value threshold to enter the model = 0.5; – p value threshold to stay in the model = 0.001

Figure 4. Relation between the weighted score of ETR RAMs and ETR FC



Median ETR FC for each category is indicated with an X; Non-virologic-failure excluded population in pooled DUET (n=406)

Table 3. Improved correlation between genotype and phenotype using the weighted 2008 ETR RAM score

	n (%)		ETR FC category, % (n) ≤3	3–13	>13
All	403 (100)	All	66.7 (269)	18.4 (74)	14.9 (60)
ETR RAM list 2007 (13 RAMs)	343 (85)	0–2	72.5 (251)	15.6 (54)	11.0 (38)
	60 (15)	≥3	30.0 (18)	33.3 (20)	36.7 (22)
Weighted ETR RAM score 2008 (17 RAMs)	225 (56)	0–2	92.4 (208)	7.1 (16)	0.4 (1)
	101 (25)	2.5–3.5	41.6 (42)	31.0 (31)	27.7 (28)
	77 (19)	≥4	24.7 (19)	35.1 (27)	40.3 (31)

- The 2008 weighted ETR RAM score provides better phenotypic prediction than the 2007 ETR RAM list, especially for the isolates with a score of 0–2

Figure 5. Relation between the weighted score of ETR RAMs and ETR FC

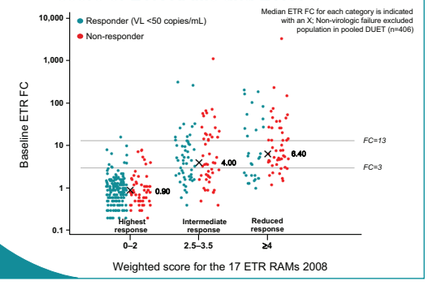
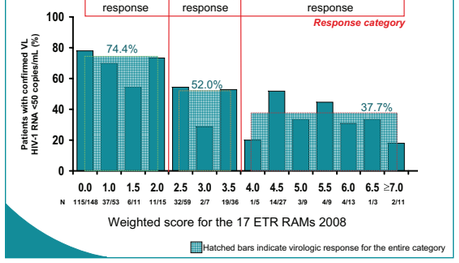


Figure 6. Relation between the weighted score and the virologic response (<50 copies/mL)



Conclusions

- A comprehensive analysis of baseline resistance data from DUET-1 and DUET-2 identified four additional mutations resulting in a list of 17 ETR RAMs: V90I, A98G, L100I, K101E/H/P, V106I, E138A, V179D/F/T, Y181C/I/V, G190A/S, and M230L
- The updated list of ETR RAMs and the relative weighting improved the relationship between genotypic and phenotypic susceptibility interpretations
- Among the 17 ETR RAMs, Y181I and Y181V had the highest weight, followed by L100I, K101P, Y181C and M230L
- Among the 17 ETR RAMs, mutations with the highest weight had a low prevalence
- The virologic response was a function of the number and weight of the baseline ETR RAMs
- A weighted mutation score of 0–2, 2.5–3.5, and ≥4 corresponded to response rates of 74% (highest response), 52% (intermediate response) and 38% (reduced response), respectively
- This new genotypic interpretation system provides better guidance in the interpretation of ETR susceptibility

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