

# Contribution of Non-Thymidine Analog Nucleoside RT Inhibitor Associated Mutations (non-TA NAMs) to Phenotypic Hypersusceptibility (HS) to Efavirenz (EFV)

12th Conference on Retroviruses and Opportunistic Infections  
February 22-25, 2005  
Boston, MA

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## Background

- EFV HS has been associated with superior clinical outcomes in NRTI-experienced subjects treated with EFV.
- Recent studies have highlighted the differences in the activity and resistance profiles of a variety of thymidine analog (TA)-sparing nucleoside analog combinations with and without EFV.
- Shulman et al. have described an association of EFV HS with T215Y, H208Y, and V118I in RT (AIDS 18: 1781, 2004)
- We evaluated the impact of non-TA NAMs on EFV susceptibility among clinical isolates in the ViroLogic database.

## Methods

- EFV HS was defined as a fold-change (FC) in  $IC_{50}$  < 0.4 using the PhenoSense assay.
- A subset of samples in the ViroLogic database which had no NNRTI mutations (A98G, L100I, K101EP, K103NS, V106AM, Y181X, Y188X, G190X, P225X, F227X, M230L, P236L or the combination of K103R+V179D), TAMs (M41L, D67N, K70R, L210W, T215FY, K219X; X=any non-wt amino acid), Q151M, or T69 insertions was analyzed.
- Isolates were identified with unmixed NRTI mutations (K65R, T69X, L74IV, V75X, M184V); there were insufficient samples with L74IV only (4) or V75X only (9) for meaningful comparisons.
- Isolates without NRTI, NNRTI or PI mutations served as a wild type (WT) reference group. Further comparator groups comprised isolates with only three TAMs including T215Y ( $\pm$  H208Y,  $\pm$  V118I) or three TAMs including K70R. Comparisons were made by a one-way ANOVA with Bonferroni's correction for multiple comparisons
- Scatter plots and statistical tests were generated using Prism 4.0 (GraphPad, San Diego, CA)

## Results Summary

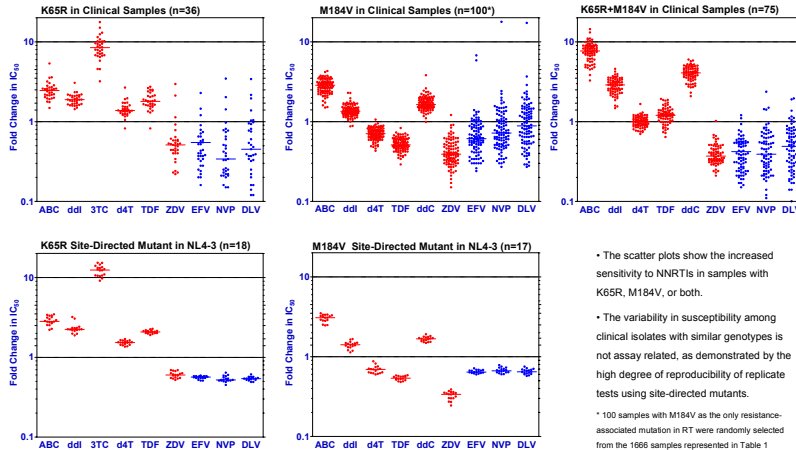
- Compared to the WT population the mean EFV FC was lower in all groups containing non-TA NAMs or TAMs ( $P < 0.05$ ; Table 1).
- Over 40% of samples with K65R, K65R + M184V, or 3 TAMs including T215Y had EFV FC < 0.4 (Table 1 and Figures 1 and 2).
- Comparisons between samples with 3 TAMs (including T215Y) with and without H208Y were not significant for any NNRTI. Also, comparisons between samples with 3 TAMs (including T215Y) with and without V118I were not significant for any NNRTI (Table 1).

**Table 1. Patterns of NNRTI Susceptibility in Samples with Non-TA NAMs**

RT Mutations	n	EFV				NVP				DLV			
		mean	median	min - max	% < 0.4	mean	median	min - max	% < 0.4	mean	median	min - max	% < 0.4
None	10,090	1.0	0.9	0.1 - 21.9	4.2%	1.1	0.9	0.1 - 52.7	8.1%	1.4	1.2	0.04 - 79	4.9%
1 NAM	1847	0.7*	0.6	0.1 - 6.8	17.8%	0.9*	0.7	0.1 - 34.1	18.0%	1.1*	0.8	0.05 - 29.4	13.2%
2 NAMs	133	0.5*	0.5	0.1 - 2.6	39.8%	0.6*	0.5	0.1 - 3.1	39.8%	0.8*	0.6	0.1 - 5.1	30.1%
3 NAMs	39	0.6*	0.5	0.2 - 1.9	28.2%	0.7	0.6	0.2 - 1.9	17.9%	0.8	0.6	0.1 - 3.0	12.8%
69x	106	0.8**	0.7	0.2 - 4.8	19.8%	0.9	0.7	0.1 - 10.2	20.8%	1.2	0.9	0.1 - 10.8	13.2%
K65R	36	0.5*	0.4	0.2 - 2.3	47.2%	0.5	0.4	0.1 - 3.5	55.6%	0.7**	0.5	0.1 - 3.4	44.4%
M184V	1666	0.7*	0.6	0.1 - 6.8	16.9%	0.9*	0.7	0.1 - 34.1	16.3%	1.1*	0.8	0.1 - 29.4	12.3%
65R-184V	75	0.4*	0.4	0.2 - 1.2	54.7%	0.5*	0.4	0.1 - 2.4	50.7%	0.6*	0.5	0.1 - 1.9	42.7%
3 TAMs incl K70R	84	0.7**	0.5	0.2 - 4.2	31.0%	0.8	0.5	0.1 - 8	36.9%	1.6	0.8	0.1 - 32.2	15.7%
3 TAMs incl T215Y	80	0.4*	0.4	0.1 - 1.3	55.0%	0.7	0.5	0.2 - 4.4	31.3%	0.4*	0.3	0.1 - 4.0	71.8%
3 TAMs incl T215Y, H208Y	11	0.4**	0.3	0.2 - 0.5	54.5%	0.6	0.6	0.4 - 0.9	9.1%	0.3	0.3	0.2 - 0.4	90.9%
3 TAMs incl T215Y, H208Y wt	69	0.4*	0.4	0.1 - 1.3	55.1%	0.7	0.5	0.2 - 4.4	34.8%	0.4*	0.3	0.1 - 4.0	68.7%
3 TAMs incl T215Y, V118I	32	0.4*	0.3	0.1 - 1.3	78.1%	0.7	0.5	0.2 - 4.4	37.5%	0.4**	0.2	0.1 - 4.0	83.9%
3 TAMs incl T215Y, V118I wt	48	0.5*	0.4	0.1 - 1.2	39.6%	0.7	0.7	0.2 - 1.5	27.1%	0.4*	0.3	0.1 - 0.9	63.8%

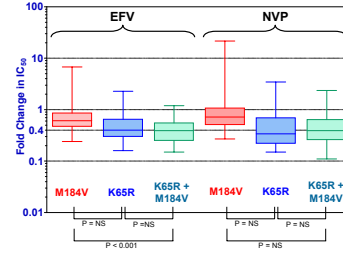
\* P<0.001 vs. no RT mutations. \*\* P<0.05 vs. no RT mutations (one-way ANOVA with Bonferroni's correction for multiple comparisons)

**Figure 1: Distribution and Median of NRTI (red) and NNRTI (blue) Fold Change Values for Clinical Isolates and Site Directed (SDMs) with M184V and/or K65R**

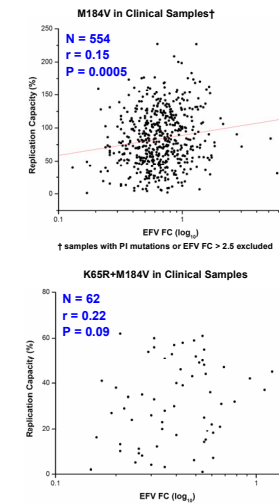


- The scatter plots show the increased sensitivity to NRTIs in samples with K65R, M184V, or both.
- The variability in susceptibility among clinical isolates with similar genotypes is not assay related, as demonstrated by the high degree of reproducibility of replicate tests using site-directed mutants.
- 100 samples with M184V as the only resistance-associated mutation in RT were randomly selected from the 1666 samples represented in Table 1

**Figure 2: Box Plots of EFV and NVP FC Distributions for M184V/I, K65R and K65R + M184V/I\***



**Figure 3: Relationship Between RC and EFV Fold Change**



## Results Summary (cont.)

- NNRTI susceptibilities were measured in site-directed mutants (SDMs) in an NL4-3 background (Figure 1). SDMs bearing only K65R had FC for EFV, NVP and DLV of  $0.56 \pm 0.02$ ,  $0.53 \pm 0.05$  and  $0.54 \pm 0.03$ , respectively (mean  $\pm$  SD of 18 replicates,  $P < 0.0001$  versus reference). SDMs bearing only M184V had FC for EFV, NVP and DLV of  $0.65 \pm 0.03$ ,  $0.67 \pm 0.05$  and  $0.65 \pm 0.05$ , respectively (mean  $\pm$  SD of 17 replicates,  $P < 0.0001$  versus reference). Although discrete these differences in FC between K65R and M184V were significant ( $P < 0.0001$ ). These data highlight the high reproducibility of the PhenoSense assay while the juxtaposed data from clinical isolates emphasize the much broader range of phenotypic susceptibilities encountered in clinical isolates with the same resistance mutations (Figure 1).
- Regression analyses comparing RC and EFV FC among isolates with M184V and no PI mutations demonstrated a very modest positive correlation ( $r=0.15$ ,  $P=0.0005$ , Figure 3). No such relationship was observed among isolates with K65R+M184V (Figure 3) or those with 3 TAMs (including T215Y) + M184V and no PI mutations ( $P=0.5$ ).

## Conclusions

- EFV HS is associated with nonTA NAMs in addition to TAMs.
- The prevalence of EFV HS with 1 or 2 nonTA NAMs is comparable to that seen with more complex patterns such as multiple TAMs + M184V.
- Among clinical isolates in the ViroLogic database, significantly enhanced EFV susceptibility is observed among viruses bearing the specific non-TA NAMs K65R, T69X, M184V and particularly the combination K65R-M184V.
- These observations were validated in NL4-3 SDMs bearing K65R or M184V which demonstrated significantly lower mean FC to EFV, NVP and DLV than WT.
- These findings may have relevance to the differing virologic outcomes and resistance profiles seen when nonTA-NRTI combinations are used with or without EFV.
- The relative contribution of different NRTI mutations to NNRTI HS merit further study.

## Acknowledgements

- We thank the ViroLogic Clinical Reference Laboratory for performing the phenotypic and genotypic resistance assays, and to Kay Limoli, Jeannette Whitcomb, and Linda Kiss for assistance with the SDM construction and assays.