

Comparison of Phenotypic Effects of NRTI Mutations on Emtricitabine (FTC) and Lamivudine (3TC)

Introduction

In treating the antiretroviral experienced, HIV-infected patient population, the impact of drug resistance associated mutations on subsequent therapy is important to consider. For patients with prior drug exposure, differences in phenotype may be difficult to predict from genotype. The algorithms used to predict drug activity may not be well defined, especially for newly approved drugs for a variety of reasons, as mutations that impact response but are selected by other antivirals may be underrepresented in virus from the initial patient population in the pivotal clinical trials.

FTC (emtricitabine) and 3TC (lamivudine) are structurally related nucleoside inhibitors of HIV-1 reverse transcriptase, and both are approved for use in treatment of HIV-infection.¹ Both drugs can select for the M184I/V mutation resulting in high levels of reduced susceptibility to either drug (>100 fold).² However, while the phenotypic susceptibility of 3TC to viruses containing common NRTI mutations is well characterized, the phenotypic susceptibility of FTC is less well understood. A large database was used to examine the resistance profiles of both drugs and the potential cross-resistance conferred by common NRTI resistance-associated mutations

Methods

The resistance profiles data was derived from HIV-infected patient plasma samples that had previously been analyzed by ViroLogic, Inc for HIV protease and RT mutations and for phenotypic resistance. The PhenoSense™ HIV assay (ViroLogic Inc, South San Francisco, CA), was used to measure drug susceptibility. Results were expressed as the median of the IC50 fold-change (clinical isolate IC50 divided by IC50 of simultaneously tested drug-sensitive reference virus [NL4-3]) values (Median Fold Change; MFC).

Reduced susceptibility for 3TC and FTC were defined by fold-change values above the cutoff. Cutoffs for 3TC and FTC are as given on the PhenoSense GT report and were defined based upon virologic outcome data from clinical trials for 3TC³, and by extrapolation from 3TC data for FTC. For quantitative analyses, samples with 3TC or FTC IC50 above the highest drug concentration tested in the assay were assigned an arbitrary fold change value of 200.

The following mutations were considered to be NRTI resistance associated mutations, and samples with these mutations were excluded unless they were part of a specific query: M41L, K65R, D67N, T69X, K70R, L74X, V75A/M/S/T, Y115F, Q151M, M184I/V/T, L210W, T215F/Y, K219X.

X = any non-wild type amino acid. Samples with mixtures at the positions in the query were excluded. Thymidine analogue mutations (TAMs) were defined as follows: M41L, D67N, K70R, L210W, T215F/Y, K219E/H/N/Q/R.

The sample groupings analyzed included those with M184I/V only, those without M184I/V but containing either: 2-3 TAMs from M41L/L210W/T215F/Y, 2-3 TAMs from D67N/K70R/K219X, any 3-4 TAMs, any 5-6 TAMs, samples with K65R, and those with T69 insertions (with TAMs allowed).

- Student t-test comparison of means was performed for groups with 10 or more samples for data on both drugs to determine whether changes were statistically significant.

Results

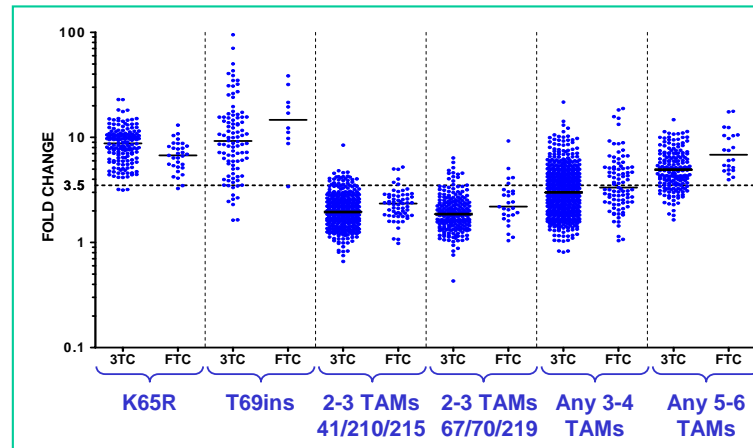
- The median fold change and the percentage of samples above the phenotypic clinical cutoff (3.5 fold) are shown for each drug in Table 1 with respect to each of the mutational groupings. As the MFC for FTC and 3TC was always above the maximum measurable level for those samples that contained M184I or V mutations, that data is not shown.
- For K65R, both 3TC and FTC had median fold changes above the 3.5 fold clinical cutoff (8.8 vs. 6.7, respectively), with almost all isolates (93.5 vs. 98.1%, respectively) being phenotypically resistant to either drug.

Table 1. Median fold changes in phenotypic susceptibility to 3TC or FTC in clinical virus samples with specific NRTI mutations, and the percentage of samples that had fold changes above the phenotypic cutoff in each group examined.

mutations	FTC			3TC		
	N	MFC	% over cutoff	N	MFC	% over cutoff
65R only	31	6.7	93.5%	161	8.8	98.1%
T69ins (TAMs allowed)*	10	14.7	90.0%	96	9.2	89.6%
2 or 3 TAMs from 41/210/215*	61	2.3	9.8%	450	1.9	6.2%
2 or 3 TAMs from 67/70/219*	27	2.2	18.5%	227	1.9	6.2%
Any 3-4 TAMs*	89	3.3	49.4%	645	3.0	38.8%
Any 5-6 TAMs*	22	6.8	100.0%	183	4.9	79.2%

*The median fold changes were significantly different (P<0.001) for the two drugs in each of these groups.

Figure 1. Scatter plots detailing fold changes in phenotypic susceptibility to 3TC or FTC for each of the clinical virus samples in each group. The median fold change for each group is shown as a bar. The 3.5 fold clinical cut-off is represented as a dashed line.



- As seen graphically in Figure 1, with respect to groups where TAMs were allowed, the median fold change was higher for FTC than 3TC in every TAM category examined, although in some cases these median values were below the 3.5 fold cut-off. The median fold changes noted for the two drugs were significantly different (P<0.001) for all TAMs containing groups.
- Similarly, in each mutational group where TAMs were allowed, more individual patient virus samples were resistant (above the 3.5 fold cutoff) to FTC than 3TC. While this effect was noted for each TAMs-containing group, the impact was greatest for virus with 2 or 3 TAMs with mutations at 67, 70 or 219. In this group, there were 3-fold more virus samples that were resistant to FTC than to 3TC.

- Reproducibility of phenotypic susceptibility measurements with this assay using site-directed NRTI mutants (presented in poster 704), suggest that the spread in the data is due mostly to genetic variability of clinical isolates.

References

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- Skowron G, J. Whitcomb, M. Westley, C. Petropoulos, N. Hellmann, et al. 1999. *Antivir Ther* 4 (suppl 1):S5-66.
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Discussion

The phenotypic profiles for both FTC and 3TC were analyzed to determine whether there were differences in susceptibility in the presence of mutations selected by other NRTIs.

Virus samples that contained the M184I or V mutations could not be compared as they exceeded upper assay limits for both drugs. With respect to K65R, the MFC for both drugs were above the clinical cutoffs, suggesting that this mutation may impact efficacy of either drug. The MFC was slightly higher for 3TC than FTC (8.8 VS 6.7), and in both cases almost all of the isolates were resistant to either drug (<5% difference in susceptibility).

More resistance was observed for FTC than 3TC in all analyses that included any grouping of TAMs, and the difference in resistance was significant (p<0.001) for all groups. These differences were not apparent when analyzing for differential phenotypic responses using the clinical cut-off. These results were similar to another analysis where virus samples containing one or more TAMs were analyzed with respect to whether they were above the 3.5 fold cutoff for FTC or 3TC. 91% concordance was seen, but when analyzed with respect to MFC, higher levels of resistance were seen for FTC than 3TC (2.6 MFC vs. 2.2 MFC, respectively, n=188).⁴

For any TAMs-containing analysis, a greater percentage of subjects retained phenotypic susceptibility to 3TC than FTC. Similar results were also noted in another analysis of virus containing TAMs from patient samples, with more cross resistance above the clinical cutoff to FTC (39%) than 3TC (30%) noted.⁴

Conclusions

- Slightly more resistance was observed for FTC than 3TC in all analyses that included any grouping of TAMs (2 or 3 TAMs from 41/210/215, 2 or 3 TAMs from 67/70/219, any 3-4 TAMs, any 5-6 TAMs, or T69ins with TAMs allowed). This difference in resistance was significant (p<0.001) for all groups.
- When looking at patient samples within any of the TAMs-containing groupings, more subjects retained phenotypic susceptibility to 3TC than FTC. The impact was most striking for subjects with virus containing 2-3 TAMs from the 67/70/219 group, where approximately three times as many subjects were resistant to FTC than 3TC.
- These results highlight that while there are some similarities with respect to response to some mutations, there are also small but significant differences in the MFC observed for FTC as compared to 3TC with respect to some specific commonly detected NRTI mutations.
- Since the clinical cut-off for FTC was inferred from that for 3TC and not derived, the clinical significance of these differences and their impact on the treatment of ART-experienced patients needs to be assessed in prospective clinical trials.