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K65R, L74V/I and M184V/I Mutations are Associated with Hypersusceptibility to 1st and Next Generation NNRTIs

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BACKGROUND

- Hypersusceptibility (HS) to first generation NNRTIs, efavirenz (EFV), nevirapine (NVP) and delavirdine (DLV), is associated with mutations selected by NRTIs and has been linked to improved virologic outcomes.[1-4]
- Although previous studies have highlighted the association of thymidine analog mutations (TAMs) with NNRTI HS, recent expansion of ART options may reduce the prevalence of TAMS in the clinic.
- This work evaluates the impact of thymidine-sparing NRTI mutations ("non-TAMs") on 1st and next generation NNRTI susceptibility.

METHODS

- Drug susceptibility (fold change (FC) in IC50 relative to wildtype) was determined using the PhenoSense assay. Hypersusceptibility was defined as FC<0.4.
- Effects of isolated non-TAMs on NNRTI susceptibility were also examined in clinical specimens from the Monogram commercial database with unmixed NRTI mutation(s) and without other resistance mutations. Only a limited number of clinical specimens with isolated L74V/I was identified (n=20).
- Clinical specimens without recognized NRTI, NNRTI or PI resistance mutations served as a wildtype (WT) reference group. Median FC of subgroups was compared to WT group.
- Site-directed mutants (SDMs) K65R, M184V, and L74V were constructed in NL4-3 and evaluated.
- Statistical analysis was performed with the statistical package R (www.r-project.org) and Prism 5.0 (GraphPad, San Diego, CA). Comparison of fold change distributions was assessed by Mann Whitney U and the Fishers Exact Test was used for hypersusceptibility comparison. Scatter plots were generated by Prism 5.0.

Table 1: Effects of Non-TAM NRTI Mutations on NNRTI Susceptibility in Clinical Specimens

		ZDV (WT=23517)			EFV (WT=23519)			NVP (WT=23519)			ETR (WT=10487)			
RT Mutation(s)	N	% HS (FC < 0.4)	Median FC	IQR	% HS (FC < 0.4)	Median FC	IQR	% HS (FC < 0.4)	Median FC	IQR	N	% HS (FC < 0.4)	Median FC	IQR
K65R	71	16.9**	0.6**	0.5 - 0.8	31.0**	0.5**	0.4 - 0.8	40.9**	0.5**	0.3 - 0.8	12	8.3#	0.7**	0.5 - 0.9
M184V/I	3762	54.4**	0.4**	0.3 - 0.5	15.9**	0.6**	0.5 - 0.9	20.6**	0.7**	0.4 - 1.0	1296	13.0**	0.7**	0.5 - 0.9
L74V/I	20	55.0**	0.4**	0.3 - 0.6	20.0*	0.8**	0.4 - 2.0	25.0*	0.9#	0.3 - 2.1	5	20.0#	0.9#	0.4 - 1.4
M184V/I + K65R	194	27.3**	0.5**	0.2 - 0.6	47.4**	0.4**	0.3 - 0.6	45.9**	0.4**	0.3 - 0.6	55	27.3**	0.5**	0.4 - 0.7
M184V/I + L74V/I	76	76.3**	0.3**	0.2 - 0.4	22.4**	0.6**	0.4 - 0.7	38.2**	0.5**	0.3 - 0.8	30	23.3**	0.6**	0.4 - 0.8

RT, reverse transcriptase; HS, hypersusceptibility; FC, fold change; IQR, interquartile range;
For all comparisons of mutant FC distributions to wildtype: **, P<0.0001; *, P<0.05; #, P not significant

Table 2: Site-Directed Mutants: Effects of K65R, M184V or L74V on NNRTI Susceptibility

		Median Fold Change**							
SDM		ABC	FTC	3TC	TFV	ZDV	EFV	NVP	ETR
K65R	FC	2.8	9.9	11.2	2.1	0.6	0.7	0.5	0.7
	IQR	2.6 - 3.0	9.3 - 10.9	10.5 - 11.6	1.9 - 2.3	0.6 - 0.7	0.6 - 0.7	0.5 - 0.6	0.7 - 0.7
M184V	FC	3.0	off scale	off scale	0.5	0.4	0.8	0.7	0.8
	IQR	2.9 - 3.2	ND	ND	0.5 - 0.6	0.3 - 0.4	0.7 - 0.8	0.7 - 0.8	0.7 - 0.8
L74V	FC	2.0	1.3	1.6	0.7	0.6	0.8	0.7	0.9
	IQR	1.8 - 2.2	1.2 - 1.4	1.2 - 1.7	0.6 - 0.8	0.5 - 0.7	0.8 - 0.8	0.6 - 0.8	0.8 - 0.9

SDM, site-directed mutant; FC, fold change; IQR, interquartile range; ND, not done
**, P<0.0001 for all comparisons of SDM FC distributions to wildtype

Figure 1a: K65R in Clinical Samples

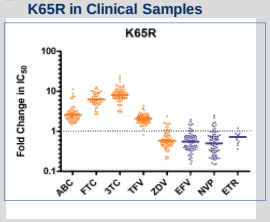


Figure 2a: M184V/I in Clinical Samples

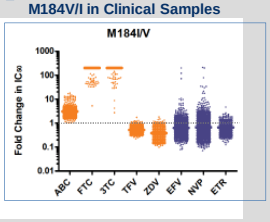


Figure 3a: L74V/I in Clinical Samples

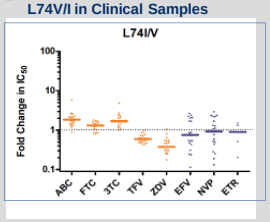


Figure 4: M184V/I + K65R in Clinical Samples

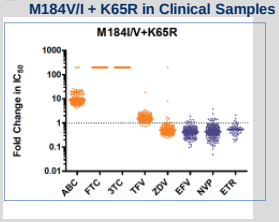


Figure 1b: K65R Site-Directed Mutant

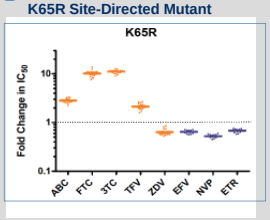


Figure 2b: M184V Site-Directed Mutant

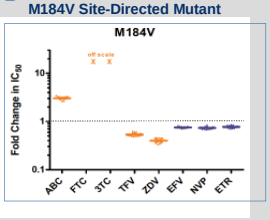


Figure 3b: L74V Site-Directed Mutant

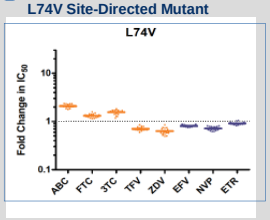
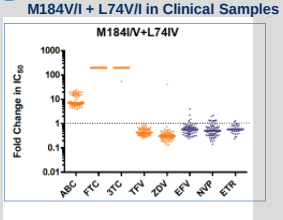


Figure 5: M184V/I + L74V/I in Clinical Samples



RESULTS

- Clinical specimens containing isolated mutations K65R, M184V/I or L74V/I demonstrated significantly increased susceptibility to EFV, NVP and the next generation NNRTI, ETR. NNRTI hypersusceptibility (FC < 0.4) in particular is frequently seen in clinical isolates with these resistance motifs (Table 1).
- Clinical samples with M184V/I, and with either K65R or L74V/I, demonstrated robust reductions in FC and high proportions with hypersusceptibility to 1st and next generation NNRTIs (Table 1, Figures 1a, 2a, 3a, 4, 5).
- Analyses of the L174V/I mutation was limited to a small number of clinical specimens bearing this mutation in isolation (n=20 for EFV, NLV; N=5 for ETR). Similarly, only 12 clinical specimens with isolated K65R were tested against ETR (Table 1).
- All SDMs were tested in 20 independent experiments. Compared to wildtype virus, K65R, M184V and L74V exhibited significantly reduced FC to 1st and next generation NNRTIs (Table 2; Figs 1b, 2b, 3b).
- Strong sensitizing effects for ZDV were also noted in SDMs and, particularly, in clinical specimens bearing the resistance motifs studied herein.

CONCLUSIONS

- Non-TAM mutations K65R, L74V/I and M184V/I are associated with reduced fold change and hypersusceptibility to first and next generation NNRTIs.
- These findings were validated in site-directed mutants and in clinical specimens from the Monogram database.
- NNRTI hypersusceptibility may have relevance for the design and success of 1st or 2nd line ART regimens, particularly those containing double NRTI therapy with a 1st or next generation NNRTI.

ACKNOWLEDGEMENTS

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