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The HIV-1 Protease Resistance Mutation I50L is Associated with Increased Susceptibility to PIs other than Atazanavir

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Background

- The HIV-1 protease inhibitor (PI) atazanavir has a distinct genotypic and phenotypic resistance profile relative to other PIs. It has been previously demonstrated[1] that the protease substitution I50L is a primary mutation encoding resistance to atazanavir.
- Analysis by site-directed mutagenesis[5] in the laboratory strain NL4-3 and three clinical isolates revealed that the presence of I50L led to increased susceptibility for 6 PIs (other than atazanavir).
- Biochemical and biophysical studies[6] revealed that presence of I50L led to increased affinity for protease by PIs other than atazanavir.
- We previously presented the prevalence of I50L between 1998 and 2005 in a large genotypic database and the impact of I50L alone and in combination with 1 to 5 primary PI mutations on predicted PI susceptibility[3].
- Here we report the further study of a large genotypic database for isolates containing the protease mutation I50L. By analyzing the mutational profiles of these sequences, we extend the characterization of I50L. We examined its contribution to clinically relevant resistance and predicted susceptibility to PIs, including the previously uncharacterized inhibitors darunavir and tipranavir.

Methods

- We examined routine clinical isolates received at Virco (excluding clinical trials) between 1999 and 2006 for the presence of I50L. These isolates were paired with other sequences in the database having like patterns of primary PI mutations as defined by IAS-USA[2]. The isolates could have other secondary PI mutations that were not considered in this analysis.
- Using vircoTYPE (ver. 4.1), the median predicted FC value for each mutational pattern was determined for all PIs, in the absence and presence of I50L respectively. For each mutational profile, a ratio of the median predicted FC (in presence of I50L: in absence of I50L) was determined for all PIs.
- I50L-related shifts in predicted FC were evaluated using relevant PI-specific ritonavir-boosted Clinical Cut Offs (CCOs) and 3 possible resistance categories: Maximal Response, Reduced Response or Minimal Response. A change across two categories in resistance was deemed Major and a change across one category was deemed Minor.

Results

Table 1. Ratio of Median predicted FC for each mutational profile against all PIs in presence and absence of protease mutation I50L.

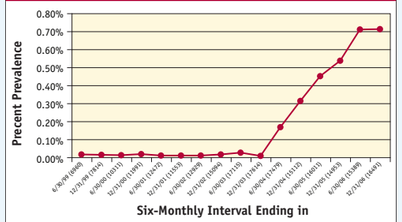
PROFILE	N =		Ratio of Median FC (I50L+ / I50L–)							
	50L+	50L–	APV/r	ATV/r	DRV/r	IDV/r	LPV/r	NFV	SQV/r	TPV/r
30N 33F 46I 50L 82A	4	5	0.4	2.7	0.6	0.3	0.1	0.4	0.2	1.1
30N 46I 50L	3	397	1.0	17.2	1.0	0.4	0.4	1.0	0.7	2.0
30N 50L	10	6742	1.7	11.2	0.8	0.6	0.6	0.7	0.7	1.2
32I 33F 46I 47V 50L 82A	8	20	0.4	3.4	0.3	0.2	0.3	0.1	0.1	1.0
32I 33F 46I 50L 82A 90M	3	73	0.6	6.6	0.4	0.2	0.3	0.5	0.2	1.2
32I 46I 47V 50L 82A	5	138	0.4	6.3	0.6	0.3	0.2	0.5	0.4	1.0
32I 46I 47V 50L	6	91	0.8	19.4	0.7	0.4	0.5	1.0	1.0	2.1
33F 46I 50L 82A 90M	11	266	0.4	4.8	0.5	0.2	0.1	0.4	0.2	0.6
33F 46I 50L 82A	12	171	0.5	3.8	0.4	0.2	0.3	0.3	0.3	0.7
33F 46I 50L 82T 90M	3	9	0.5	3.8	**0.2**	0.4	0.2	0.3	0.2	**0.2**
33F 46I 50L 90M	9	49	0.4	3.7	0.4	0.2	0.1	0.2	0.2	1.0
33F 46L 50L 82A 90M	3	338	0.6	3.1	0.4	0.1	0.2	0.3	0.3	0.9
33F 46L 50L 82A	9	300	**0.3**	2.7	0.4	0.1	0.1	0.2	0.1	1.3
33F 50L 82A 90M	6	606	0.9	1.5	0.6	0.1	0.1	0.1	0.1	0.7
33F 50L 82A	5	273	0.3	1.2	0.4	**0.04**	**0.04**	**0.1**	**0.1**	0.6
33F 50L 88S	3	3	0.6	16.6	0.5	0.6	0.4	0.8	1.0	1.0
33F 50L 90M	5	161	0.6	7.5	0.5	0.3	0.1	0.3	0.6	0.9
33F 50L	13	338	1.3	**35.3**	0.8	0.7	0.6	1.5	0.7	1.2
46I 50L 82A 90M	25	833	0.8	2.9	0.6	0.2	0.1	0.3	0.2	0.7
46I 50L 82A	18	1135	0.6	5.1	0.4	0.3	0.3	0.3	0.5	0.9
46I 50L 88S	5	353	1.0	11.6	0.7	0.2	0.4	0.3	0.9	1.4
46I 50L 90M	25	3276	0.8	12.7	0.6	0.4	0.4	0.5	0.6	1.0
46I 50L	15	992	0.7	14.4	0.7	0.4	0.4	0.4	0.6	1.2
46L 50L 82A 90M	7	1001	1.1	8.7	0.7	0.3	0.6	0.7	0.7	1.0
46L 50L 82A	16	1455	0.7	4.5	0.6	0.1	0.1	0.2	0.4	0.9
46L 50L 90M	4	384	1.4	21.3	0.8	0.4	0.8	0.8	0.7	1.2
46L 50L	3	708	0.7	6.9	0.4	0.4	0.5	0.3	0.5	0.8
50L 82A 90M	7	2625	0.6	5.4	0.5	0.2	0.1	0.3	0.3	0.7
50L 82A	15	2672	0.6	5.0	0.4	0.2	0.1	0.2	0.2	0.8
50L 88S	8	576	0.5	8.7	0.7	0.3	0.4	0.5	0.5	1.0
50L 90M	41	11108	0.9	18.3	0.7	0.6	0.6	0.7	1.0	1.0
TOTAL N=	307	37098								

Bolded numbers indicate maximum resistance or susceptibility

Table 2. The Major and Minor shifts in Resistance Calls due to I50L-related decrease in median FC among the mutational profiles analyzed (N=31).

Protease Inhibitor	Major Shift (2-category change)	Minor Shift (1-category change)
Atazanavir/r	12.9% (4/31)	71% (2 2/31)
Darunavir/r	0	6.5% (2/31)
Fosamprenavir/r	0	22.6% (7/31)
Indinavir/r	0	48.4% (15/31)
Lopinavir/r	6.5% (2/31)	41.9% (13/31)
Nelfinavir	3.2% (1/31)	45.2% (14/31)
Saquinavir/r	3.2% (1/31)	19.4% (6/31)
Tipranavir/r	0	19.4% (6/31)

Figure 1. The percent prevalence of protease mutation I50L among routine clinical isolates received at Virco, shown by six-monthly intervals between 1999 and 2006.



The numbers in parenthesis on the X axis indicate the number of isolates received in each six-month interval

- The prevalence of I50L increased from 0.01% in the period up to 2003 (prior to atazanavir's approval) to 0.7% in 2006 (*Figure 1*).
- We examined a total of 224,813 routine clinical isolates of which 474 contained I50L, corresponding to 380 unique mutation profiles. Of these, 457 and 41,001 samples with and without I50L respectively (comprising 66 mutation profiles) had one or more concurrent primary PI mutations as defined by IAS-USA[2].
- A further selection of a minimum of ≥3 samples in each profile yielded 307 and 37,098 samples with and without I50L respectively (N=31 mutation profiles).
- The presence of I50L caused decreased susceptibility to atazanavir in all 31 mutational contexts as the ratio of predicted FC (+I50L:-I50L) was >1 (*Table 1*).
- The predicted FC ratio for other PIs revealed that a majority of the mutational patterns demonstrated I50L-related higher susceptibility as follows: amprenavir (25/31), darunavir (30/31), indinavir (31/31), lopinavir (31/31), nelfinavir (28/31), saquinavir (29/31) The predicted FC ratio for mutational patterns resulting in higher susceptibility for tipranavir was relatively modest: 13/31, while 8/31 showed no change (*Table 1*).
- The largest I50L-associated shifts in median predicted FC were: atazanavir (1.2 to 42.4), amprenavir (10.2 to 3.2), darunavir (3.3 to 0.5), indinavir (13 to 0.5), lopinavir (34.9 to 1.3), nelfinavir (22.3 to 1.3), saquinavir (5.2 to 0.3), and tipranavir (29.9 to 5.2). A graph of representative mutational profiles exhibiting the largest shift for indicated PIs and a scatter plot of every isolate in the same mutation profile is shown in *Figure 2*.
- Clinical relevance for the median predicted FC value of each mutational pattern was provided by comparison to drug-specific CCO values. An evaluation of the I50L-related shifts in resistance categories revealed that, a varied number of the mutational patterns (range: 6.5% for darunavir to 71% for atazanavir) caused a minor shift across one-resistance category. A major shift across two resistance categories was observed across a smaller range (12.9% for atazanavir to 3.2% for nelfinavir/saquinavir (*Table 2*)).

Figure 2. Fold change observed for all PIs in the presence and absence of I50L, and scatter plot of individual isolates for the displayed mutational profile

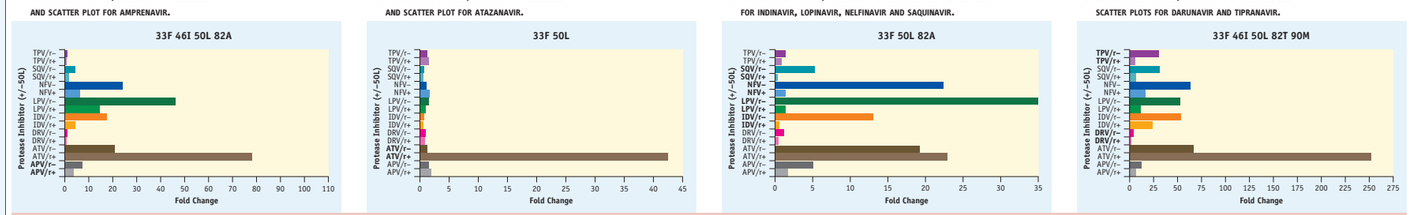
Fold change (FC) observed for all PIs in the presence (+) and absence (–) of I50L for the indicated mutational profile.

A. Mutational profile, maximum FC observed and scatter plot for amprenavir.

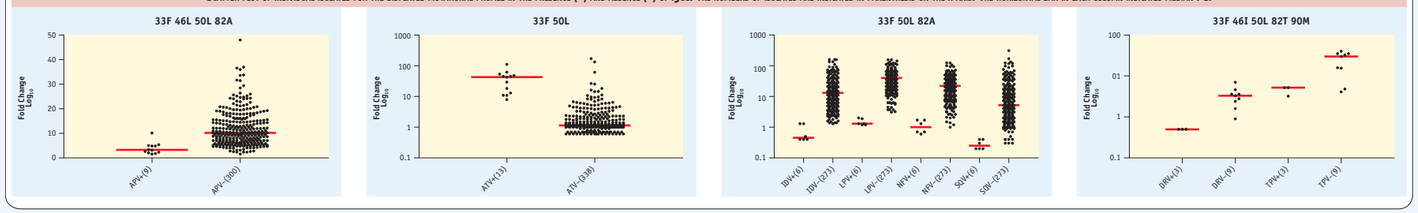
B. Mutational profile, maximum FC observed and scatter plot for atazanavir.

C. Mutational profile, maximum FC observed and scatter plots for indinavir, lopinavir, nelfinavir and saquinavir.

D. Mutational profile, maximum FC observed and scatter plots for darunavir and tipranavir.



Scatter plot of individual isolates for the displayed mutational profile in the presence (+) and absence (–) of I50L. The numbers of isolates are indicated in parenthesis on the x axis. The horizontal bar in each column indicates median FC.



Discussion

- The PI atazanavir was approved by various regulatory health authorities beginning in June 2003. The subsequent rise in prevalence of the resistance mutation I50L is consistent with the increased availability of this PI. The prevalence of I50L in our database appears to have reached a plateau at 0.7% in 2006
- Previous studies concluded that I50L confers resistance to atazanavir and susceptibility to other PIs[1,5,6]. Since these conclusions were based on studies in a very limited number of laboratory and clinical isolates, their clinical implications were not clear[5].
- The linear model which is the underlying engine for vircoTYPE predictions[4], assigned Resistance Weight Factors (RWF) to I50L such that it conferred resistance to atazanavir and varying degrees of susceptibility to other PIs.
- For comparison, a similar analysis of the sensitizing effect of the reverse transcriptase mutation M184V on sensitivity to zidovudine was evaluated. An examination of 566 mutational profiles in presence and absence of M184V revealed that while the presence of M184V caused a minor or one-category shift in sensitivity to zidovudine for 30% of the mutational profiles, no profiles showed a major or two-category shift in sensitivity (data not shown).
- Our analyses extend and confirm previous observations on I50L-associated increased susceptibility of PIs other than atazanavir, in a large database of routine clinical isolates.

Conclusion

- The presence of PI mutation I50L is associated with resistance to atazanavir and increased susceptibility to other PIs in a variety of protease mutational backgrounds.

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