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Short communication

The HIV-1 protease resistance mutation I50L is associated with resistance to atazanavir and susceptibility to other protease inhibitors in multiple mutational contexts

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Abstract

Background: The HIV-1 protease mutation I50 L causes atazanavir resistance but increases susceptibility to other PIs. Predicted phenotypic FC values were obtained from viral genotypes, using the virtual Phenotype-LM bioinformatics tool (powering vircoTYPE).
Objective: To evaluate I50 L's effect on susceptibility to 8 PIs, in a large genotype database.
Study design: I50 L containing routine clinical isolate samples in Virco's genotype database were paired with samples having like patterns (or profiles) of IAS-USA-defined primary PI mutations, but lacking I50 L. Using vircoTYPE (version 4.1), the median predicted FC for each mutational profile was determined. I50 L-associated shifts in FC were evaluated using drug-specific CCOs.
Results: We selected 307 and 37098 samples with and without I50 L. These corresponded to 31 mutation patterns of ≥ 3 samples each. I50 L caused resistance to atazanavir in all 31 mutation contexts, but was associated with higher susceptibility for other PIs. The largest I50 L-associated shifts in median predicted FC were: 1.2 to 42.4 (atazanavir), 10.2 to 3.2 (amprenavir), 3.3 to 0.5 (darunavir), 13 to 0.5 (indinavir), 34.9 to 1.3 (lopinavir), 22.3 to 1.3 (nelfinavir), 5.2 to 0.3 (saquinavir) and 29.9 to 5.2 (tipranavir).
Conclusions: The PI mutation I50 L causes clinically relevant resistance and increased susceptibility to atazanavir and other PIs respectively.

Keywords: I50L; Atazanavir; PI hyper-susceptibility; Predicted phenotypic resistance; Clinically relevant resistance; HIV-1 resistance

1. Introduction

The availability of the PI class of inhibitors enabled the beginning of HAART era for HIV-1 infected patients. The PI atazanavir has a distinct genotypic and phenotypic resistance profile relative to other PIs. Colonno et al. (2004) demonstrated that the protease substitution I50L encodes resistance to atazanavir. Site-directed mutagenesis studies by Weinheimer et al. (2005) showed that presence of I50L led to increased susceptibility for six PIs. Furthermore biochemical and biophysical studies by Yanchunas et al. (2005) revealed that presence of I50L led to increased affinity for protease by other PIs. We previously reported I50L prevalence (0.54% in 2005) in Virco's genotypic database (Sista et al., 2006). Analysis of I50L alone and in combination with (IAS-USA-defined) 1–5 primary PI mutations revealed notable phenotypic resistance (predicted FC > CCO1) for amprenavir and nelfinavir, while susceptibility to indinavir, lopinavir, tipranavir and saquinavir was maintained (Sista et al., 2006).

Historically, analysis of HIV-1 resistance in patients failing therapy is analyzed by either genotypic or phenotypic methods, with relative benefits for both (Van Laethem and Vandamme, 2006). A hybrid approach where predicted phenotype is obtained from a genotype, using a correla-

Abbreviations: PI, protease inhibitor; RWF, resistance weight factor; FC, fold change; CCO, clinical cut-off; ARV, anti-retroviral; HAART, highly active anti-retroviral therapy.

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tive geno/pheno database and linear regression modeling was described recently (Vermeiren et al., 2007). In this approach, the phenotypic susceptibility is estimated as the weighted sum of the effects of individual mutations as well as higher order interactions terms (mutation pairs). This method accounts for both synergistic and antagonistic interactions between mutations. Furthermore, clinical relevance is provided by the establishment of drug-specific CCOs that are derived using a combination of baseline resistance data and a clinical outcomes database (Winters et al., in press).

We report the 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 by examining its contribution to clinically relevant resistance and susceptibility to PIs, including the previously uncharacterized PIs darunavir and tipranavir.

2. 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 sequences in the database having like patterns of the following (IAS-USA) primary PI mutations: 30N, 32I, 33F, 46I, 46L, 47A, 47V, 48V, 50L, 50V, 54L, 54M, 76V, 82A, 82F, 82S, 82T, 84V, 88S, 90M (Johnson et al., 2006). Although the isolates could have other secondary PI mutations that were not considered in this analysis, their contribution to the net predicted FC was accounted for and included in the linear model.

Using vircoTYPE (ver. 4.1), PI FC values were predicted for each genotype. The median predicted PI FC value for each mutational pattern was determined in the absence and presence of I50L and the ratio of the median FCs (in absence of I50L:in presence of I50L) was calculated (for profiles $\geq$3 isolates).

I50L-related shifts in FC were evaluated using PI-specific ritonavir-boosted CCOs and three possible resistance categories: maximal, reduced or minimal response. A change across two categories in resistance was deemed Major and a change across one category was deemed Minor.

3. Results

Prevalence of I50L increased from 0.01% in the period up to 2003 (prior to atazanavir's approval) to 0.71% in 2006 (Fig. 1). Among the approximately 224,000 routine clinical isolate sequences received between 1999 and 2006 at Virco, 474 sequences contained I50L. A majority ($N = 457$) had other IAS-USA primary PI mutations (Johnson et al., 2006). Starting with a matched subset of isolates that contained like patterns of primary PI mutations, we identified 307 and 37,098 sequences with and without I50L, respectively. These isolates comprised 31 mutational patterns of 3 or more sequences each (Sista et al., 2007).

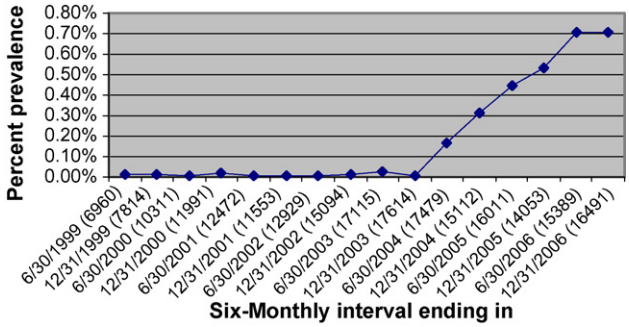

Fig. 1. The percent prevalence of protease mutation I50L among routine clinical isolates received at Virco, shown by 6-monthly intervals between 1999 and 2006. The numbers in parenthesis on the *X*-axis indicate the number of isolates received in each 6-month interval.

Presence of I50L caused decreased susceptibility to atazanavir in all 31 mutational contexts (FC ratio > 1 (+I50L:−I50L)) (Table 1). For other PIs, an examination of the FC ratio 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) (see Table 1). For tipranavir, the number of mutation profiles exhibiting I50L-associated resistance and susceptibility were 10/31 and 13/31, respectively, with 8/31 showing no change. The largest I50L-associated shifts in median 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 0.9), nelfinavir (22.3 to 1.3), saquinavir (5.3 to 0.3), and tipranavir (29.9 to 5.2).

Clinical relevance for the predicted median FC value of each mutational pattern was provided by drug-specific CCOs. An inspection of I50L-related shifts in resistance categories revealed that shifts across one or more resistance categories was observed for all PIs. Of the 31 mutational patterns evaluated, the following number of patterns exhibited shifts (Major/Minor) in resistance categories: fosamprenavir/r (0/5), atazanavir/r (4/22), darunavir/r (0/2), indinavir/r (0/15), lopinavir/r (2/13), nelfinavir (1/14), saquinavir/r (1/6) and tipranavir/r (0/3).

4. 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 genotype database appears to have reached a plateau at 0.74% in 2006. One explanation may be stabilization in the rate of failure among patients taking atazanavir-containing regimens, though other explanations are also possible.

Previous studies concluded that I50L confers resistance to atazanavir and susceptibility to other PIs (Colonno