

Prevalence of Darunavir Resistance—Associated Mutations: Patterns of Occurrence and Association with Past Treatment

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(See the editorial commentary by Haubrich, on pages 1125–7.)

Eleven protease mutations have been associated with reduced susceptibility to darunavir (DRV). We examined the prevalence and covariates of these mutations in 2 populations. Thirty percent of 1175 Northern California patients and 24% of 2744 non-California patients in the Stanford HIV Drug Resistance Database had viruses with 1 or more mutations associated with resistance to DRV. In multivariate analyses, the number of DRV resistance-associated mutations depended on the number of previous protease inhibitors (PIs) administered and on amprenavir/fosamprenavir treatment. Most PI-treated patients should respond favorably to DRV-based salvage therapy.

Darunavir (DRV, formerly TMC114) is a recently licensed protease inhibitor (PI) with in vitro activity against HIV-1 isolates resistant to other PIs and with clinical efficacy in the treatment of persons in whom multiple previous PI-containing regimens have failed [1, 2]. Much of the DRV-prescribing information is derived from the phase II POWER registration studies, in which different ritonavir-boosted doses of DRV, as well as a

comparison ritonavir-boosted dose of PI, were used for salvage therapy [3].

In an adjunctive analysis of the POWER studies, De Meyer et al. identified, at 10 protease positions, 11 mutations associated with both a reduced in vitro susceptibility to DRV and a reduced in vivo virologic response to DRV salvage therapy [4]. Among subjects receiving DRV/r, the percentages of subjects with 0, 1 or 2, and 3 or more of the 11 DRV resistance-associated mutations who attained plasma HIV-1 RNA levels <50 copies/mL at week 24 were 60%, 45%, and ≤20%, respectively [4]. Although other covariates, such as baseline plasma HIV-1 RNA levels and the use of enfuvirtide, influenced virologic response, no PI resistance-associated mutations other than the 11 identified by De Meyer et al. were reported to influence the virologic response.

The prevalence of these 11 DRV resistance-associated mutations in PI-naïve and PI-treated patients, the risk factors for the development of these mutations, and the other protease mutations with which these mutations occur have not been described. Therefore, we examined their prevalence and covariates in 2 mutually exclusive populations with different potential biases: patients in a large representative US clinic population and patients in published studies in the Stanford HIV Drug Resistance Database.

Patients, materials, and methods. HIV-1 protease sequences were examined in 2 mutually exclusive populations with a known history of PI treatment: (1) a population of PI-treated patients from 16 clinics of Kaiser-Permanente Medical Care Program—Northern California, from whom plasma samples were collected and submitted for genotypic resistance testing at Stanford University Hospital between 1998 and 2006, and (2) PI-treated patients described in published studies in the Stanford HIV Drug Resistance Database [5]. For viruses undergoing genotypic resistance testing at Stanford University, treatment histories were obtained from patients' charts and pharmacy records, as part of a collaboration approved by the institutional review board. For data from published studies, treatment histories were supplemented with requests for information from the studies' authors.

DRV resistance-associated mutations were defined by De Meyer et al. as the following 11 differences, at 10 protease positions, in the subtype B consensus protease sequence: V11I, V32I, L33F, I47V, I50V, I54L, I54M, G73S, L76V, I84V, and L89V [4]. Mutations that were present as part of an electrophoretic mixture (i.e., more than 1 peak was present at a position on the sequence electropherogram) were classified as

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Table 1. Prevalence of 11 darunavir resistance-associated mutations in 2 mutually exclusive populations of protease inhibitor-treated individuals.

Mutation	Stanford HIV Drug Resistance Database (n = 2744)	Northern California clinic (n = 1175)
V11I	1.0	2.3
V32I	2.8	5.0
L33F	3.0	7.3
I47V	1.3	2.6
I50V	1.2	1.4
I54L/M	1.8	5.8
G73S	6.5	8.3
L76V	1.1	1.7
I84V	8.6	13.0
L89V	2.9	1.9

NOTE. Data are % of individuals.

mutations. Sequences containing a mixture of I54L and I54M, however, were considered to have only 1 DRV resistance-associated mutation at position 54. If more than 1 virus isolate with DRV resistance-associated mutations was taken from a patient, the isolate with the most DRV resistance-associated mutations was used to determine the frequency and patterns of DRV resistance-associated mutations.

Results. The clinic population included 1847 patients, of whom 1175 had received 1 or more PIs. The database population included 11,697 patients, of whom 2744 had received 1 or more PIs. Each of the 11 DRV resistance-associated mutations was uncommon among sequences from PI-naïve individuals, having rarely occurred at a prevalence of >0.5% in any of the 8 most common subtypes ([5], <http://hivdb.stanford.edu/cgi-bin/MutPrevBySubtypeRx.cgi>). Notable exceptions include V11I, which was present in 0.6%, 1.9%, 0.6%, and 2.3% of CRF01_AE, CRF02_AG, D, and G isolates, respectively, and L33F, which was present in 1.0% and 1.2% of A and AE isolates, respectively. We note that L33V and other substitutions at position 89 (particularly L89M) were highly polymorphic, with L89M being the consensus residue in multiple subtypes.

In contrast to their almost complete absence in isolates from PI-naïve persons, the 11 DRV resistance-associated mutations did occur in PI-treated persons. Among the 1175 Northern California patients, 29.8% had 1 or more DRV resistance-associated mutations, including 25.7% who had 1 or 2 mutations and 4.1% who had 3–6 mutations. Among the 2744 patients in the Stanford HIV Drug Resistance Database, 23.7% had 1 or more DRV resistance-associated mutations, including 22.8% who had 1 or 2 mutations and 0.9% who had 3–6 mutations. Plasma HIV-1 RNA levels and CD4 counts were available for the 1175 Northern California patients. Among the 350 patients

with DRV resistance-associated mutations, the median RNA level was 4.3 log copies/mL, and the median CD4 count was 220 cells/mm³. Among the 825 PI-treated patients without DRV resistance-associated mutations, the median RNA level was 4.0 log copies/mL, and the median CD4 count was 272 cells/mm³.

I84V, G73S, L33F, V32I, I54L, and I54M were the most common mutations in both populations (table 1); V11I, I47V, I50V, L76V, and L89V were the least common. The most common combinations of mutations included G73S+I84V, L33F+I84V, V32I+I47V, L33F+I54L/M, and I54L/M+I84V.

Among the 1175 PI-treated clinic patients, 37%, 24%, 17%, and 22% received 1, 2, 3, and 4 or more PIs, respectively. Among the 2744 PI-treated database patients, 62%, 19%, 12%, and 8% received 1, 2, 3, and 4 or more PIs, respectively. The proportions of patients receiving each of the PIs were similar in both data sets. Among the pooled clinic and database patients, indinavir was used in 53%, nelfinavir in 48%, saquinavir in 34%, lopinavir in 9%, amprenavir and/or fosamprenavir in 8%, atazanavir in 2%, and tipranavir in 0.4%.

For the clinic population and the database population, individually, we created several multivariate regression models to characterize the relationship between past antiretroviral therapy

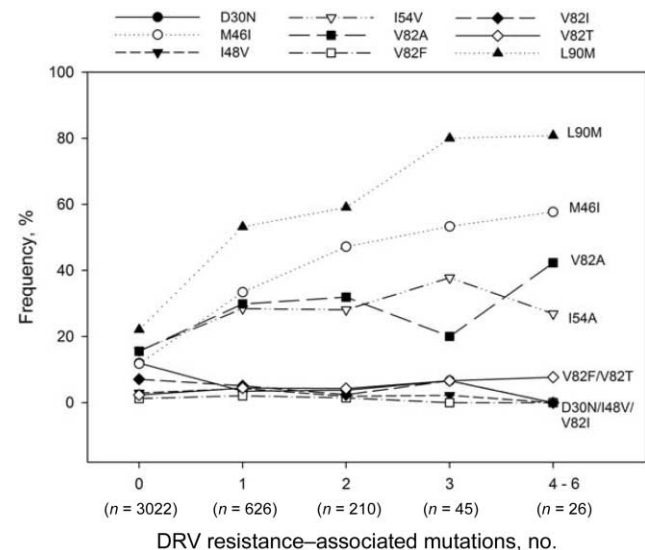


Figure 1. Correlation between number of darunavir (DRV) resistance-associated mutations and 9 additional PI resistance-associated mutations. The x-axis represents the no. of DRV resistance-associated mutations (with the *n* values, in parentheses, indicating the no. of patients), and the y-axis represents the frequency of the 9 PI resistance-associated mutations: D30N, M46I, I48V, I54V, V82A, V82F, V82I, V82T, and L90M. M46I and L90M were the only mutations that increased in frequency as the number of DRV resistance-associated mutations increased. M46I and L90M were significantly correlated with the number of DRV resistance-associated mutations, even after we controlled for multiple comparisons. None of the remaining mutations were positively correlated with the number of DRV resistance-associated mutations. The database and clinic population sequences were pooled for this analysis.

and the number of DRV resistance-associated mutations. In both populations, the number of DRV resistance-associated mutations was independently positively associated both with the number of PIs previously received ($P < 10^{-16}$) and with having previously received either amprenavir or fosamprenavir ($P < 10^{-16}$). When we controlled for the number of PIs received, there was a negative association with having received nelfinavir ($P < 10^{-4}$), in both populations, and with having received atazanavir ($P < 10^{-5}$), in the clinic population.

Because PI resistance-associated mutations often occur in complex patterns, we performed a regression analysis to identify mutations that increased in prevalence with an increasing number of DRV resistance-associated mutations and that might therefore also be predictors of response to DRV therapy. For this analysis, we included only sequences containing at least 1 of the following nonpolymorphic PI resistance-associated mutations: D30N, V32I, M46I/L, I47A/V, G48V, I50L/V, I54A/V/L/M, L76V, V82A/F/L/S/T, I84V, N88D/S, and L90M, to exclude mutations that might appear to be associated with DRV resistance-associated mutations simply because they were more common in isolates with evidence of selective drug pressure.

A total of 131 mutations at 62 protease positions were present in viruses from 15 or more treated patients in the data set of pooled clinic and database patients. After using the method of Benjamini and Hochberg [6], to control the false-discovery rate at 0.05, we found that 17 mutations at 16 protease positions were significantly associated with the number of DRV resistance-associated mutations, including L10I ($P < 2e^{-16}$), M46I ($P < 2e^{-16}$), A71V ($P < 2e^{-16}$), I72L ($P < 2e^{-16}$), L90M ($P < 2e^{-16}$), C67F ($P = 3.5e^{-11}$), G16A ($P = 3.93e^{-11}$), L63P ($P = 8.5e^{-11}$), K55R ($P = 6.8e^{-9}$), K43T ($P = 7.8e^{-9}$), F53L ($P = 9.8e^{-8}$), G73C ($P = 1.4e^{-7}$), I62V ($P = 8.0e^{-7}$), G73T ($P = 3.9e^{-6}$), I85V ($P = 2.2e^{-5}$), L10F ($P = 2.0e^{-4}$), and Q18H ($P = 4.0e^{-4}$). Figure 1 shows the results of the analysis for 9 of the most common non-DRV PI resistance-associated mutations, demonstrating the positive correlation with M46I and L90M but not with D30N, G48V, I54V, and V82A/F/I/T.

Discussion. Because the DRV resistance-associated mutations are not among the most commonly occurring PI resistance-associated mutations, we sought to identify their prevalence, their patterns of co-occurrence, and their risk factors in a clinic population and in an online database of protease sequences from published studies. Our results show that 96% of PI-treated persons in a Northern California clinic population and 99% of PI-treated persons listed in the database have fewer than 3 DRV resistance-associated mutations and would therefore be expected to have a favorable response to DRV, with an ~50% chance of achieving a plasma HIV-1 RNA level of <50 copies/mL by week 24 [4].

Not surprisingly, both the number of previously received PIs and previous amprenavir or fosamprenavir treatments were as-

sociated with an increased number of DRV resistance-associated mutations. Amprenavir and DRV are highly similar molecules, the only difference being that DRV has a fused bicyclic tetrahydrofuran [7]. Moreover, the drug resistance profiles are similar; 9 of the 11 DRV resistance-associated mutations also reduce amprenavir susceptibility [8].

Several lines of evidence suggest that the list of DRV resistance-associated mutations reported by De Meyer et al. may not be the only mutations influencing susceptibility to DRV and virologic response. First, attempts at selecting for resistance to DRV were unsuccessful in vitro when they began with the wild-type virus [1] but not when they began with viruses containing other PI resistance-associated mutations [9]. Second, mutations are often surrogates for other drug resistance-associated mutations. The rapid emergence of resistance to newly administered PIs has been reported to develop in patients having a pretherapy plasma virus that contains multiple PI resistance-associated mutations but that nonetheless is susceptible to the prescribed PI [9]. Finally, the clinical data supporting the current list of DRV resistance-associated mutations are based on a single-patient population. Common mutations such as M46I and L90M, which we found to be highly correlated with the number of DRV resistance-associated mutations, may have an effect on virologic outcome despite not having had a significant effect when added to a model that already contained 11 mutations with which they were highly correlated.

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