Mutations associated with virological response to darunavir/ritonavir in HIV-1-infected protease inhibitor-experienced patients

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Objective: The aim of the study was to identify a pattern of protease gene mutations associated with the virological response to darunavir/ritonavir-based regimens. Patients and methods: We analysed 153 treatment-experienced patients receiving a darunavir/ritonavir salvage regimen as a sole protease inhibitor (PI). Virological response was defined as an HIV-1 RNA load of <200 copies/mL at month 3. The impact of individual protease gene mutations on the virological response to darunavir/ritonavir was examined, and the combination of mutations most strongly associated with the virological response was identified.

Results: The baseline median HIV RNA level was 4.7 log10 copies/mL and the median CD4 cell count was 142 cells/mm3. At month 3, 55% of patients had a virological response and the median fall in viral load from baseline was 1.7 log10 copies/mL. All the patients had detectable darunavir concentrations at month 3. Cochran–Armitage procedure identified eight mutations with a negative impact on the virological response, namely K14R, K20I, E34Q, I47V, I54M, K55R, T74P and I84V; and two mutations (E35D and V82A) with a positive impact. In multivariate analyses, our genotypic scores were highly predictive of the virological response at month 3, along with the baseline plasma viral load and enfuvirtide co-prescription to enfuvirtide-naive patients.

Conclusions: Among the eight mutations with a negative impact on the virological response, I47V, I54M, T74P and I84V were previously described as darunavir resistance-associated mutations. Some PI resistance mutations had a positive impact on the virological response. These findings might help to explain the potency of darunavir/ritonavir on PI-resistant HIV.

Keywords: resistance, genotypic score, genotype interpretation

Introduction

Darunavir (formerly TMC114) is a recently licensed protease inhibitor (PI) with in vitro activity against both wild-type and PI-resistant HIV-1 isolates and has exhibited clinical efficacy in patients in whom multiple PI-containing regimens have failed.1,2 Darunavir has potent activity, with a 50% effective concentration of 1–5 nM for HIV-1.1 Modelling and crystallographic studies of the HIV-1 protease have demonstrated unusual darunavir binding characteristics that predict greater resilience to the development of resistance and greater activity against resistant viruses than earlier PIs.1–6 In vitro selection of darunavir-resistant HIV-1 from wild-type strains (HIV-1LAI/ NL4-3/IIIIB) appears to be slower and less frequent than with
other PIs, likely reflecting the particularly strong binding of darunavir to the HIV protease. The efficacy of ritonavir-boosted darunavir combined with an optimized background regimen has been studied in highly treatment-experienced HIV-infected patients in two Phase IIb trials; virological and immunological efficacy were superior to those of investigator-selected control PIs plus optimized background regimen. It is now widely recognized that studies correlating the virological response in treatment-experienced patients to the baseline genotypic profile provide useful information for the development of drug-specific resistance algorithms. The available data on genotypic resistance patterns predicting virological response to darunavir/ritonavir in PI-experienced patients are limited to two studies. The objective of this study was to determine, using a previously validated method and outcome data, a pattern of mutations predictive of virological response to darunavir/ritonavir in PI-experienced patients.

Patients and methods

Patients and antiretroviral regimens

This study was a retrospective analysis of genotypic resistance tests, HIV-1 plasma RNA and CD4 cell counts, which were performed in patients failing antiretroviral therapy just before receiving a darunavir/ritonavir-containing regimen, as recommended by the French HIV guidelines for the use of antiretroviral agents in HIV-1-infected patients in the context of routine clinical practice (http://www.sante-jeunesse-sports.gouv.fr/publications-documentation/publications-documentation-sante/rapports/rapport-du-groupe-experts-2008-prise-charge-medicale-patients-infectees-par-vih-sous-direction-du-pr-patrick-k-yni.html). Patients were eligible for the study if they had had at least 6 months of PI exposure, had received a darunavir/ritonavir-containing regimen and had a plasma HIV-1 RNA load >500 copies/mL at baseline. Patients receiving other PIs in addition to darunavir/ritonavir were excluded, as were patients who had a treatment interruption before switching to darunavir/ritonavir. All patients had detectable darunavir in plasma at month 1 and month 3. One hundred and fifty-three PI-experienced patients were studied retrospectively. All were prescribed highly active antiretroviral therapy regimens containing darunavir (600 mg twice daily) and ritonavir (100 mg twice daily). The background regimen consisted of nucleoside reverse transcriptase inhibitors (NRTIs) and/or non-nucleoside reverse transcriptase inhibitors (NNRTIs) and/or enfuvirtide. Participating laboratories belonged to the ANRS AC11 network and participated regularly in the ANRS quality-control assessment of HIV-1 drug resistance sequencing.

Plasma HIV-1 RNA assay

HIV-1 RNA was determined with the Cobas Amplicor HIV-1 Monitor test, version 1.5 (Roche Diagnostics, Basel, Switzerland) in plasma samples collected at baseline and at 3 months or 6 months. All protease and RT gene mutations were identified from the International AIDS Society resistance testing USA panel (September 2008).

The genotypic sensitivity score (GSS) was determined from the results of baseline resistance genotypic tests. We calculated the GSS that represents the sum of genotypic sensitivities according to the ANRS AC-11 genotype interpretation algorithm (www.hivfrenchresistance.org): 0, 0.5 or 1 if resistant, partially susceptible or susceptible to the drugs co-prescribed with darunavir/ritonavir in the new regimen, respectively. Enfuvirtide was considered as an active drug only when prescribed in enfuvirtide-naive patients.

Darunavir assay in plasma

Steady-state trough concentrations of darunavir were determined in plasma samples collected at months 3–6 by using a specific validated high-performance liquid chromatography assay coupled with fluorescence detection after solid–liquid extraction. The lower limit of quantification was 5 ng/mL. The within-day and between-day coefficients of variation were below 10% in quality-control plasma samples.

Statistical methods

The main end-point was the percentage of virological responders at month 3. A virological response was defined as plasma HIV RNA <200 copies/mL corresponding to the lower limit of quantification in both virology laboratories. The relationship between individual baseline protease mutations (codons 1–99) and virological response was studied with Fisher’s exact test. Mutations present in at least 10% of patients and with \( P \) values <0.20 in univariate analysis were analysed with a removal procedure and a non-parametric test to select the combination of mutations most strongly associated with virological response. The Cochran–Armitage (CA) test was used. The removal procedure begins with all \( k \) mutations selected by univariate analysis. The first step is to compute the \( P \) value with the CA test corresponding to a score including the initial \( k \) mutations. One by one, all combinations of \( k \) mutations are tested and the combination providing the lowest \( P \) value in the CA test is retained if the \( P \) value is below that obtained with the \( k \) mutations. Mutations are then again removed one by one to compare the combinations of \( k – 1 \) mutations. The combination of mutations providing the lowest \( P \) value, lower than the \( P \) value obtained with \( k – 1 \) mutations, is again retained, and so on. The procedure ends when removing a mutation does not provide a lower \( P \) value than the previous one. As some individual mutations were associated with a better virological response, we repeated this analysis and obtained a second mutation score taking into account both favourable and unfavourable mutations. To assess whether or not the genotypic score was an independent predictor of the virological response, we used backward multivariate logistic regression including baseline variables associated with the virological response \((P<0.10)\) among the following: treatment history, treatment co-prescribed with darunavir/ritonavir, baseline viral load, CD4 cell count, number of darunavir/ritonavir co-prescribed active drugs (GSS) and the HIV-1 subtype. SAS statistical software version 9.1 was used.

Results

Characteristics of the patients

All the 153 patients had detectable darunavir plasma concentrations in the target range and were retrospectively studied to establish the genotypic resistance score. The main characteristics of the patients...
of the study population are shown in Table 1. At baseline, the median plasma HIV-1 RNA load was 4.7 log_{10} copies/mL [interquartile range (IQR): 4.3–5.2] and the median CD4 cell count was 142 cells/mm^3 [IQR: 28–264]. Prior to this study, the patients had been exposed to a median of 12 antiretroviral drugs (range: 10–14), including a median of 6 NRTIs (IQR: 5–7) and 4 PIs (IQR: 3–5). The PIs previously received were indinavir/ritonavir (82% of patients), saquinavir/ritonavir (78%), amprenavir/ritonavir (78%), lopinavir (73%), nelfinavir (61%), tipranavir/ritonavir (39%) and atazanavir/ritonavir (14%). At baseline, the median numbers (IQR) of major and minor PI resistance mutations, based on the International AIDS Society-USA (IAS-USA) list, were 4 (3–8) and 9 (7–10), respectively.12 The frequency of each PI mutation at baseline is shown in Figure 1. Major PI resistance mutations at codons 33, 46, 82, 84 and 90 were the most frequently observed (>50%). The median RT inhibitor GSS of the study regimens was 1 (range: 0–5) according to the 2007 ANRS algorithm.

Impact of PI resistance mutations on the virological response

Respectively, 84 (55%) and 87 (56%) of the 153 patients had a virological response to the darunavir/ritonavir-containing regimen at month 3 and at month 6. The median drop in the plasma HIV RNA level from baseline was 1.68 log_{10} copies/mL (IQR: 2.2 to 2.34) at month 3 and 1.69 log_{10} copies/mL (IQR: 2.41 to 2.0) at month 6.

Table 2 shows the influence of mutated and wild-type codons at specific sites of the protease gene on virological response, as determined by univariate analysis. Mutations at 10 codons (14R, 20I, 34Q, 47V, 54M, 55R, 74P, 84V, 89V and 90M) were associated with a lower rate of virological response to darunavir/ritonavir at month 3 (P<0.20). L54M and I84V are listed as major darunavir resistance mutations on the IAS-USA list. Two other major darunavir resistance mutations (I50V and L76V) were not retained by univariate analysis, owing to a prevalence below 10% [I50V, n=10 (6.5%) or to a P value >0.20 [L76V, n=21 (13.7%); P=0.25]. Mutations K20R, E35D and V82A were associated with a higher rate of virological response. The other variables associated with the virological response at month 3 were the baseline viral load (P<0.0001), the baseline CD4 cell count (P=0.015), the number of IAS major PI mutations at baseline (≤4 versus >4, P=0.009) and enfuvirtide co-prescription to enfuvirtide-naive patients (P=0.029). The number of active drugs in the darunavir/ritonavir-containing regimens, based on the GSS, did not influence the virological response (P=0.143). The previous use of any PIs was not associated with virological response.

**Darunavir/ritonavir genotypic scores**

Removing procedure. Among the mutations with a negative impact on the virological response in univariate analysis, the CA removal procedure did not select the mutations at codons 89 and 90, therefore, the following genotypic darunavir score was obtained (darunavir/ritonavir score 1): 14R, 20I, 34Q, 47V,
Table 2. Amino acid substitutions in HIV-1 protease associated with a lower rate of virological response to darunavir/ritonavir-containing regimen (univariate analysis)

<table>
<thead>
<tr>
<th>Protease codon</th>
<th>Prevalence of individual mutation at baseline</th>
<th>Non-respondersa</th>
<th>Respondersa</th>
<th>P valueb</th>
<th>Impact of mutation on virological response</th>
</tr>
</thead>
<tbody>
<tr>
<td>K14</td>
<td>137 (89.5)</td>
<td>58 (42.3)</td>
<td>79 (57.7)</td>
<td>—</td>
<td>negative</td>
</tr>
<tr>
<td>14R</td>
<td>16 (10.5)</td>
<td>11 (68.8)</td>
<td>5 (31.3)</td>
<td>0.0621</td>
<td>negative</td>
</tr>
<tr>
<td>KMQRVT20</td>
<td>135 (88.2)</td>
<td>55 (40.7)</td>
<td>80 (59.3)</td>
<td>—</td>
<td>negative</td>
</tr>
<tr>
<td>20I</td>
<td>18 (11.8)</td>
<td>14 (77.8)</td>
<td>4 (22.2)</td>
<td>0.0045</td>
<td>negative</td>
</tr>
<tr>
<td>KIMQTV20</td>
<td>96 (62.7)</td>
<td>50 (52.1)</td>
<td>46 (47.9)</td>
<td>—</td>
<td>positive</td>
</tr>
<tr>
<td>20R</td>
<td>57 (37.3)</td>
<td>19 (33.3)</td>
<td>38 (66.7)</td>
<td>0.0292</td>
<td>positive</td>
</tr>
<tr>
<td>EADKVV34</td>
<td>135 (88.2)</td>
<td>57 (42.2)</td>
<td>78 (57.8)</td>
<td>—</td>
<td>negative</td>
</tr>
<tr>
<td>34Q</td>
<td>18 (11.8)</td>
<td>12 (66.7)</td>
<td>6 (33.3)</td>
<td>0.0757</td>
<td>negative</td>
</tr>
<tr>
<td>EGKN35</td>
<td>79 (51.6)</td>
<td>42 (53.2)</td>
<td>37 (46.8)</td>
<td>—</td>
<td>negative</td>
</tr>
<tr>
<td>35D</td>
<td>74 (48.4)</td>
<td>27 (36.5)</td>
<td>47 (63.5)</td>
<td>0.0509</td>
<td>positive</td>
</tr>
<tr>
<td>I47</td>
<td>123 (80.4)</td>
<td>49 (39.8)</td>
<td>74 (60.2)</td>
<td>—</td>
<td>negative</td>
</tr>
<tr>
<td>47V</td>
<td>30 (19.6)</td>
<td>20 (66.7)</td>
<td>10 (33.3)</td>
<td>0.0131</td>
<td>negative</td>
</tr>
<tr>
<td>IALVST54</td>
<td>129 (84.3)</td>
<td>53 (41.1)</td>
<td>76 (58.9)</td>
<td>—</td>
<td>negative</td>
</tr>
<tr>
<td>54M</td>
<td>24 (15.7)</td>
<td>16 (66.7)</td>
<td>8 (33.3)</td>
<td>0.0257</td>
<td>negative</td>
</tr>
<tr>
<td>KHINQ55</td>
<td>104 (68.0)</td>
<td>42 (40.4)</td>
<td>62 (59.6)</td>
<td>—</td>
<td>negative</td>
</tr>
<tr>
<td>55R</td>
<td>49 (32.0)</td>
<td>27 (55.1)</td>
<td>22 (44.9)</td>
<td>0.1168</td>
<td>negative</td>
</tr>
<tr>
<td>TASS74</td>
<td>128 (83.7)</td>
<td>54 (42.2)</td>
<td>74 (57.8)</td>
<td>—</td>
<td>negative</td>
</tr>
<tr>
<td>74P</td>
<td>25 (16.3)</td>
<td>15 (60.0)</td>
<td>10 (40.0)</td>
<td>0.1254</td>
<td>negative</td>
</tr>
<tr>
<td>VCEIL82</td>
<td>94 (61.4)</td>
<td>48 (51.1)</td>
<td>46 (48.9)</td>
<td>—</td>
<td>negative</td>
</tr>
<tr>
<td>82A</td>
<td>59 (38.6)</td>
<td>21 (35.6)</td>
<td>38 (64.4)</td>
<td>0.0682</td>
<td>positive</td>
</tr>
<tr>
<td>I84</td>
<td>66 (43.1)</td>
<td>24 (36.4)</td>
<td>42 (63.6)</td>
<td>—</td>
<td>negative</td>
</tr>
<tr>
<td>84V</td>
<td>87 (56.9)</td>
<td>45 (51.7)</td>
<td>42 (48.3)</td>
<td>0.0716</td>
<td>negative</td>
</tr>
<tr>
<td>LAIMST89</td>
<td>128 (84.2)</td>
<td>54 (42.2)</td>
<td>74 (57.8)</td>
<td>—</td>
<td>negative</td>
</tr>
<tr>
<td>89V</td>
<td>24 (15.8)</td>
<td>15 (62.5)</td>
<td>9 (37.5)</td>
<td>0.0768</td>
<td>negative</td>
</tr>
<tr>
<td>L90</td>
<td>58 (38.2)</td>
<td>21 (36.2)</td>
<td>37 (63.8)</td>
<td>—</td>
<td>negative</td>
</tr>
<tr>
<td>90M</td>
<td>94 (61.8)</td>
<td>48 (51.1)</td>
<td>46 (48.9)</td>
<td>0.0937</td>
<td>negative</td>
</tr>
</tbody>
</table>

aVirological response is defined as viral load ≥200 copies/mL at month 3.
bValue of Fisher’s exact test.

54M, 55R, 74P, 84V (P = 2.54 × 10⁻³). The virological response rates were, respectively, 78% (n = 46), 59% (n = 56), 36% (n = 36), 18% (n = 11) and 0% (n = 4) in patients with 0, 1, 2, 3 and ≥4 mutations (Figure 2a).

Although the major darunavir mutations I50V and L76V were not selected by univariate analysis, adding these two mutations to darunavir/ritonavir score 1 in order to include all major darunavir resistance mutations, created a new genotypic score (darunavir/ritonavir score 2)—14R, 20I, 34Q, 47V, 50V, 54M, 55R, 74P, 76V, 84V—showing a stronger association with the virological response (P = 9.04 × 10⁻²). The response rates were, respectively, 83% (n = 18), 77% (n = 40), 56% (n = 43), 38% (n = 29) and 13% (n = 23) in patients with 0, 1, 2, 3 and ≥4 mutations (Figure 2b).

We also tested the influence of all mutations with either a negative or a positive impact on the virological response in univariate analysis. This time the CA removal procedure did not select mutations K20R and L90M and yielded the following genotypic score (darunavir/ritonavir score 3): 14R, 20I, 34Q–35D, 47V, 54M, 55R, 74P–82A, 84V. 89V (P = 5.80 × 10⁻⁸). As shown in Figure 2(c), the response rate was 78% among the nine patients with a genotypic score of –2, compared with 90% (n = 20), 57% (n = 35), 66% (n = 41), 40% (n = 25), 7% (n = 15) and 12% (n = 8), respectively, in the patients with genotypic scores of –1, 0, 1, 2, 3 and ≥4. This latter score (darunavir/ritonavir score 3) was not retained for further analyses, as the P value was higher than the one observed for darunavir/ritonavir scores 1 and 2. Furthermore, the virological response distribution did not show a continuous decrease according to the number of PI mutations as observed with darunavir/ritonavir scores 1 and 2.

Impact of the HIV-1 subtype on the virological response to darunavir/ritonavir at month 3

Twenty-nine patients were infected with non-B subtype HIV-1 strains (8 A, 1 C, 4 D, 1 F, 3 G, 1 H, 1 J, 5 CRF02_AG, 1 CRF06, 2 CRF12, 1 CRF14, 1 CRF09). HIV-1 subtype was not associated with significant differences in virological response (57% for subtype B and 45% for non-B subtypes, P = 0.30).

Multivariate analysis

The following variables had P values <0.05 in univariate analysis and were included in the final multivariate models:
Darunavir/ritonavir score 1 or 2, baseline viral load, baseline CD4 cell counts, baseline IAS major PI mutations and enfuvirtide co-prescription to enfuvirtide-naive patients. In the final multivariate model, darunavir/ritonavir score 1 ($P = 0.0001$), baseline HIV RNA (log copies/mL) ($P = 0.0005$) and enfuvirtide co-prescription to enfuvirtide-naive patients ($n = 33$, OR $= 3$, $P = 0.01$) were independently associated with the virological response at month 3. Similar results were obtained with darunavir/ritonavir score 2 ($P < 0.0001$), baseline HIV RNA ($P = 0.0005$) and enfuvirtide co-prescription to enfuvirtide-naive patients ($n = 33$, OR $= 3$, $P = 0.01$) were independently associated with the virological response at month 3. Similar results were obtained with darunavir/ritonavir score 1 ($P = 0.00005$) and darunavir/ritonavir score 2 ($P = 0.00005$) were predictive of the virological response at month 6.

The darunavir/ritonavir POWER mutation score, derived from the pooled analysis of the Phase II POWER 1, 2 and 3 studies in PI-experienced patients, includes the following mutations: 11I, 32I, 33F, 47V, 50V, 54L/M, 73S, 76V, 84V and 89V. In our patient population, this mutation score was associated with virological response at month 3 ($P = 0.0002$) and month 6 ($P = 0.0005$). In contrast, the mutation score obtained in the PREDIZISTA study (13V, 32I, 33F/I/V/L, 35D, 36I/L/V, 47V/A/I, 53L, 62V) was not associated with virological response at month 3 ($P = 0.5208$) or month 6 ($P = 0.5820$).10

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**Figure 2.** Darunavir/ritonavir genotypic scores. Numbers of patients are given in parentheses after each score.

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darunavir/ritonavir genotypic scores. Numbers of patients are given in parentheses after each score.
Discussion

Genotypic resistance scores based on the assessment of the impact of genotypic patterns at baseline on the subsequent virological response can provide objective and useful guidance for antiretroviral drug selection, particularly for treatment-experienced patients. In this study including patients receiving darunavir/ritonavir-containing salvage therapy, we derived a darunavir/ritonavir genotypic resistance score by using a previously described stepwise procedure.14–17 A virological response, defined as viral load <200 copies/mL at month 3, was observed in 55% of our patients. This represents a potential limitation of our study since the recent guidelines recommend that the goal of the antiretroviral therapy is the suppression of HIV plasma RNA to <50 copies/mL.19 Changes at eight codons of the protease gene (14R, 20I, 34Q, 47V, 54M, 55R, 74P and 84V) were used to compose a genotypic resistance score (darunavir/ritonavir score 1) capable of predicting virological response to darunavir/ritonavir-containing regimens. Three of the eight mutations in darunavir/ritonavir score 1 (47V, 54M and 84V) are darunavir resistance-associated mutations based on the IAS-USA spring 2008 resistance expert list (www.iasusa.org). Recently, De Meyer et al.20 proposed a revised darunavir/ritonavir genotypic score based on a pooled analysis of the POWER and DUET (etravirine Phase III) trials, which involved a total of 1071 experienced patients. In this study including patients receiving darunavir/ritonavir-containing salvage therapy, we derived a darunavir/ritonavir genotypic resistance score by using a previously described stepwise procedure.14–17 A virological response, defined as viral load <200 copies/mL at month 3, was observed in 55% of our patients. This represents a potential limitation of our study since the recent guidelines recommend that the goal of the antiretroviral therapy is the suppression of HIV plasma RNA to <50 copies/mL.19 Changes at eight codons of the protease gene (14R, 20I, 34Q, 47V, 54M, 55R, 74P and 84V) were used to compose a genotypic resistance score (darunavir/ritonavir score 1) capable of predicting virological response to darunavir/ritonavir-containing regimens. Three of the eight mutations in darunavir/ritonavir score 1 (47V, 54M and 84V) are darunavir resistance-associated mutations based on the IAS-USA spring 2008 resistance expert list (www.iasusa.org). Recently, De Meyer et al.20 proposed a revised darunavir/ritonavir genotypic score based on a pooled analysis of the POWER and DUET (etravirine Phase III) trials, which involved a total of 1071 patients. As we did, they found that mutation 74P, which had not previously been linked to darunavir resistance, had a negative impact on the virological response to darunavir/ritonavir-based regimens.20 Mutation 73S, included in the previous darunavir/ritonavir POWER score from Tibotec, was not retained in the revised score. The V11I, V32I, L33F, I50V, I54L and L76V darunavir resistance-associated mutations could not be included in our darunavir/ritonavir resistance score 1, either because of their low prevalence (I50V and I54L) or because of a P value >0.20 in univariate analysis (V11I, V32I, L33F, I54L and L76V). However, the predictive value of darunavir/ritonavir score 1 was significantly improved when mutations I50V and L76V, which are major darunavir/ritonavir resistance mutations, were added. This amended score (darunavir/ritonavir score 2) containing all known major darunavir resistance mutations provided a better prediction of the virological response in our patient population.

Three substitutions, including the major resistance mutation V82A, were associated with a better virological response to darunavir/ritonavir. This probably explains the activity of darunavir on viruses harbouring this classic major PI resistance mutation. Our darunavir/ritonavir score 3 took into account all mutations with a positive and a negative impact on the virological response, however, it was not more predictive than scores 1 and 2 in terms of virological outcome and thus was not retained.

Compared with previously published darunavir/ritonavir GSSs, our new algorithm is both highly predictive and discriminatory with regard to the virological response to darunavir/ritonavir-containing regimens. It is not surprising that a score derived from another dataset should be less predictive when applied to our dataset, owing to the different patient populations, viruses and statistical methods used. However, the darunavir/ritonavir score derived from pooled week 24 data of the POWER 1, 2 and 3 trials was predictive of the virological response at months 3 and 6 in our patients, even though the method used to build that mutation score was different. It is noteworthy, however, that certain baseline characteristics of our patients were similar to those of the POWER trial populations, such as the duration of antiviral exposure, the number of antiretroviral drugs received before starting the darunavir/ritonavir regimen, the number of PIs received before the darunavir/ritonavir regimen, the CD4 cell count and plasma viral load.9 In contrast, the PREDIZISTA darunavir/ritonavir genotypic score was not predictive of the virological response at month 3 or month 6 in our patients.10 As the baseline characteristics of the PREDIZISTA study population were comparable to those of our study population, this discordance might be attributable to the small size of the population, which comprised only 63 patients, in the PREDIZISTA study.

In our study, as in all recent trials of antiretroviral salvage regimens, enfuvirtide co-prescription to enfuvirtide-naive patients was associated with a better virological response in multivariate analysis.21,22

It was recently reported that the virological response to a PI/ritonavir-containing regimen might be compromised in patients with non-clade B HIV-1 infection suggesting that natural amino acid variations in the protease gene, commonly named polymorphisms, might have an impact on virological response to PIs. Indeed, Marcelin et al.18 showed that the virological response to a tipranavir/ritonavir-containing regimen was lower in PI-experienced patients infected by non-B subtype HIV-1 strains than in patients with subtype B HIV-1 infection (25% versus 59%, P=0.015), despite similar antiviral activity of tipranavir/ritonavir against non-subtype B and subtype B isolates in vitro.23 It is noteworthy that most mutations affecting the virological response to tipranavir/ritonavir are significantly more frequent in non-B subtype HIV-1 strains. Moreover, in the lopinavir/ritonavir monotherapy arm of the MONARK trial, which compared the safety and efficacy of lopinavir/ritonavir monotherapy and a standard lopinavir/ritonavir+zidovudine/lamivudine regimen in antiretroviral-naive subjects, patients infected with non-B subtype strains had worse virological responses at week 48 than patients infected with subtype B viruses.24 Multivariate analysis indicated that the HIV subtype was independently associated with virological response at week 48. In our study, the mutation K20I was associated with the worst virological response but the K20R mutation, which is more prevalent in non-B subtypes, was associated with a better virological response, and we may speculate that if this mutation is present the virological response to a darunavir/ritonavir-containing regimen should be better. However, the virological response to darunavir/ritonavir was not associated with the infecting subtype in our study. Similar findings were also recently reported in the ARTEMIS Phase III trial.25

PIs are designed to fit the active site of HIV-1 protease and are thus sensitive to structural changes in the viral protein. The binding affinity of darunavir for the wild-type HIV-1 protease is 100-fold higher than that of other PIs, owing to slower darunavir dissociation from the protease active site.26 Crystallographic studies of HIV-1 show that compared with other PIs, darunavir establishes direct interactions with residues located in the active site of the protease enzyme, and in particular, with the aspartic acid at positions 25, 29 and 30, whereas other PIs interact with side chains at the S2 subsite of the enzyme.3,27 The high activity of darunavir on the different HIV-1 subtypes, as well as on HIV-2,28 can be explained by the fact that this drug interacts strongly with residues that are important for the activity of
of the protease enzyme and thus are highly conserved among all HIV strains.

One potential limitation of our score is that all the mutations are assigned equal weights, a consequence related to the current lack of consensus on weighting methods. A large number of models can perform similarly, and external validation is thus needed to show that they apply to the majority of patients. Ultimately, this newly proposed darunavir/ritonavir resistance score should be cross-validated in a different dataset such as the POWER trial dataset. However, our darunavir/ritonavir resistance score is based on a relatively large number of patients with multiple PI resistance mutations at baseline and could thus be useful for predicting the virological response to darunavir/ritonavir in PI-experienced patients.

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Transparency declarations

None to declare.

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