Clinically validated mutation scores for HIV-1 resistance to fosamprenavir/ritonavir


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Background: We developed clinically relevant genotypic scores for resistance to fosamprenavir/ritonavir in HIV-1 protease inhibitor (PI)-experienced patients.

Methods: PI-experienced patients with virological failure receiving fosamprenavir/ritonavir as the sole PI for at least 3 months and with detectable fosamprenavir plasma levels were included. The impact of baseline protease mutations on virological response (VR, i.e. decrease in plasma HIV-1 RNA between baseline and month 3) was analysed using the Mann–Whitney test. Mutations with prevalence >10% and P value <0.10 were retained. The Jonckheere–Terpstra test was used to select the combination of mutations most strongly associated with VR. The association between score and VR was assessed by multivariate backward regression.

Results: In the 73 patients included, the median baseline HIV-1 RNA was 4.6 log10 copies/mL (range: 2.7–6.9) and the mean decrease at month 3 was −1.07 ± 1.40 log10 copies/mL. Ninety per cent of the patients were infected by HIV-1 subtype B variants. Two fosamprenavir/ritonavir mutation scores were constructed: score A (L10F/I/V → L33F → M36I → I54L/M/V/A/T/S → I84V → L90M) was based only on mutations associated with a worse VR, whereas score B (L10FIV → L33F → M36I → I54L/V/A/T/S → A71V → V77I → N88S → L90M) also took into account favourable mutations. Both scores were independent predictors of VR, however, co-administration of tenofovir was associated with a worse VR and the presence of the N88S protease mutation and co-administration of enfuvirtide with a better VR.

Conclusions: These clinically validated mutation scores should be of interest for the clinical management of PI-experienced patients. The fosamprenavir/ritonavir score A was introduced in the 2006 ANRS algorithm along with isolated mutations I50V and V32I.

Keywords: HIV-1 drug resistance, genotype, protease inhibitor

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Fosamprenavir/ritonavir mutation score

Introduction

The selection of HIV-1 drug-resistant variants is one of the major factors limiting the efficacy of antiretroviral therapy (ART).1 HIV-1 protease inhibitors (PIs) are now administered with a low-dose of ritonavir, used as a pharmacological booster. High PI plasma levels are targeted both to prevent the selection of resistant variants in ART-naive patients and to obtain antiviral efficacy in patients having viruses already carrying PI resistance mutations. In this setting, there is a need for clinically validated interpretation algorithms for genotypic resistance to boosted PIs in order to refine the indications for these drugs in PI-experienced patients.

Fosamprenavir is a prodrug of the PI amprenavir and, when boosted with ritonavir, has shown an antiviral potency comparable to lopinavir/ritonavir in treatment-naive patients.2 The protease mutations selected in vitro or in vivo in the presence of amprenavir (150V, V321 + 147V, I54L/M and 184V)3 are rarely selected in patients treated by first-line fosamprenavir-containing regimens, particularly when boosted with ritonavir.4,5

In PI-experienced patients, few data are available concerning the antiviral efficacy of fosamprenavir/ritonavir and the prediction of virological response (VR) by genotypic resistance analysis.7 We report here the identification of baseline protease mutations associated with the VR to fosamprenavir/ritonavir in PI-experienced patients, and the design of clinically relevant fosamprenavir/ritonavir mutation scores.

Patients and methods

Study population

Treatment-experienced patients with at least 6 months of prior exposure to PI who were switched to fosamprenavir/ritonavir-containing ART and with plasma HIV-1 RNA >500 copies/mL at baseline were eligible. Patients receiving other PI in addition to fosamprenavir/ritonavir or with undetectable amprenavir plasma level at month 1 or month 3 were excluded, as well as patients with treatment interruption before the switch to fosamprenavir/ritonavir. Patients switching from amprenavir/ritonavir to fosamprenavir/ritonavir were also excluded.

Patients received 700 mg fosamprenavir boosted with 100 mg ritonavir twice daily along with other antiretroviral drugs selected by the clinician based on available resistance testing. Demographic data, previous drug exposure history and current drug regimen were recorded. All patients gave written informed consent and the study was approved by the Comité Consultatif de Traitement de l’Information dans la Recherche Scientifique et Médicale and the Commission Nationale Informatique et Libertés.

Genotypic resistance analysis

Sequencing of the reverse transcriptase (RT) and protease regions was performed on plasma samples collected at baseline or <3 months before treatment switch, according to the Agence Nationale de Recherche sur le SIDA (ANRS) consensus protocol; the details of the methods appear at www.hivfrenchresistance.org. All amino acid substitutions in the protease were listed for further analysis. RT and protease resistance mutations, as listed by the International AIDS Society–USA Panel (www.iasusa.org, update October 2005), were also reported, including the distinction between major and minor PI resistance mutations.

The genotypic sensitivity score for reverse transcriptase inhibitors (RTI-GSS) was defined as the number of active RTI co-prescribed with fosamprenavir/ritonavir, using the ANRS algorithm (update July 2006) for interpretation of resistance and scoring +1 for sensitivity, +0.5 for possible resistance and 0 for resistance.

Plasma HIV-1 RNA assays

HIV-1 RNA was determined using Amplicor HIV Monitor or Amplicor HIV Cobas Taq Man (Roche, Basel, Switzerland) on plasma samples collected at baseline and at 3 months.

Determination of fosamprenavir concentrations in plasma

Steady-state trough plasma concentrations of fosamprenavir were determined on plasma samples collected at month 1 or at month 3 on fosamprenavir/ritonavir using a specific and validated high-performance liquid chromatography assay coupled with ultraviolet photodiode array detection after liquid–liquid extraction as described previously.8 The lower limit of quantification was 30 ng/mL. The coefficients of variation within-day and between-day were <10% in plasma quality controls.

Statistical analyses

VR was defined as the change in HIV-1 RNA between baseline and month 3. Associations between all baseline PI mutations and VR were analysed by the Mann–Whitney test. Mutations detected in >10% of patients and with either P value ≤0.1 or known to be selected in failing patients1–2 were retained for further analysis. We used the Jonckheere–Terpstra removing procedure to select the final set of mutations with a negative impact on VR, defining the genotypic score.9 From the initial set of k mutations retained in the first stage, all mutations were removed one by one to investigate all combinations of k–1 mutations. The Jonckheere–Terpstra test was used to compare all different combinations of mutations. The combination of k–1 mutations providing the lower P value was retained. Then, mutations were again removed one by one to compare the different combinations of k–2 mutations; the combination providing the lower P value was again retained. The procedure was repeated several times and stopped when removing a mutation did not provide a lower P value than the previous P value.

Since some individual mutations were associated with a better VR, we repeated this analysis leading to 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 response, we performed a backward multivariate linear regression accounting for baseline variables found to be associated with VR with P value <0.10 among treatment history, treatment co-prescribed with fosamprenavir/ritonavir, baseline viral load, CD4 count, RTI-GSS and HIV-1 subtype.

Results

Patients’ characteristics

After exclusion of three patients with undetectable fosamprenavir plasma levels, 73 patients were included in the analysis. The baseline characteristics are listed in Table 1. At baseline, patients had a median HIV-1 RNA of 4.6 log_{10} copies/mL.
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Table 1. Baseline patient characteristics (n = 73)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, n (%)</td>
<td>male 54 (74); female 19 (26)</td>
</tr>
<tr>
<td>Median age (years) (IQR)</td>
<td>45 (40–50)</td>
</tr>
<tr>
<td>Transmission group, n (%)</td>
<td>sex between men 34 (47); sex between men and women 22 (30); intravenous drug use 9 (12); other/unknown 8 (11)</td>
</tr>
<tr>
<td>Median number of previous ARVs (IQR)</td>
<td>all ARVs 10 (8–11); NRTIs 5 (4–6); NNRTIs 1 (1–2); PIs 3 (2–4)</td>
</tr>
<tr>
<td>Median duration of exposure to ARVs (years) (IQR)</td>
<td>NRTIs 9 (7–12); NNRTIs 6 (4–6); PIs 8 (5–8)</td>
</tr>
<tr>
<td>Median plasma HIV-1 RNA (log_{10} copies/mL) (IQR)</td>
<td>4.6 (3.8–5.0)</td>
</tr>
<tr>
<td>Median CD4 cell count (cells/mm(^3)) (IQR)</td>
<td>224 (135–425)</td>
</tr>
</tbody>
</table>

Co-prescribed ARVs, number of patients (%)

- 3TC: 36 (49)
- ABC: 30 (41)
- TDF: 32 (44)
- ddi: 21 (29)
- FTC: 15 (21)
- ZDV: 9 (12)
- d4T: 2 (3.0)
- NVP: 3 (4)
- EFV: 2 (3)
- T20: 10 (14)

RTI-GSS, number of patients (%)

- 0: 7 (10)
- 0.5: 14 (19)
- 1: 17 (23)
- 1.5: 10 (14)
- 2: 20 (27)
- ≥2.5: 5 (7)

T20 in T20-naive patients, n (%) 6 (8)

HIV-1 subtype, n (%) 66 (90)

Non-B 7 (10)

IQR, interquartile range; ARVs, antiretroviral drugs; NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, non-nucleoside reverse transcriptase inhibitors; PIs, protease inhibitors; RTI-GSS, genotypic sensitivity score of the co-prescribed reverse transcriptase inhibitors; T20 in T20-naive patients, co-prescription of enfuvirtide in patients not previously exposed to enfuvirtide; 3TC, lamivudine; ABC, abacavir; TDF, tenofovir; ddi, didanosine; FTC, emtricitabine; ZDV, zidovudine; d4T, stavudine; NVP, nevirapine; EFV, efavirenz; T20, enfuvirtide.

Patients had received unboosted amprenavir. Sixteen per cent of the patients had received enfuvirtide.

At baseline, major PI mutations were detected in 75% of the patients. The median number of major PI mutations was 2 (range: 0–4) and the median number of minor PI mutations was 5 (range: 1–7). The frequency of baseline PI mutations is shown in Figure 1. According to the ANRS version 2006 algorithm, 84% of the patients were resistant to at least one NRTI, 40% to non-nucleoside reverse transcriptase inhibitors and 84% to at least one PI. The median RTI-GSS of the new regimen was 1 (range: 0–4).

Impact of PI resistance mutations on VR

After 3 months on fosamprenavir/ritonavir, the mean decrease in plasma HIV-1 RNA was \(-1.07 \pm 1.40 \log_{10} \text{copies/mL}\) and the median decrease of \(-0.62 \log_{10} \text{copies/mL}\) [interquartile range (IQR): \(-2.23; -0.04\)]. Only 13 patients had a viral load below the limit of detection (50 copies/mL).

The mean decrease in HIV-1 plasma RNA according to the presence or absence of PI resistance mutations is shown in Table 2. All protease mutations were tested here. Substitutions at 12 codons (10, 33, 34, 36, 46, 54, 62, 71, 72, 73, 82 and 90) were associated with a worse VR, with a prevalence of over 10%. In contrast, the mutations V77I and N88S were associated with larger decreases in viral load. The association between I84V and a poorer VR did not reach significance.

Fosamprenavir/ritonavir mutation scores

Starting from the 12 mutations associated with a worse VR, plus the mutation I84V (a key mutation for resistance to amprenavir), the following combination of 8 mutations L10F/I/V, L33F, M36I, I54L/M/V/A/T/S, I62V, V82A/F/C/G, I84V, and L90M was strongly associated (Jonckheere–Terpstra test, \(P = 2.6 \times 10^{-4}\)) with the plasma HIV-1 RNA change at month 3. As shown in Figure 2(a), the mean decrease in HIV-1 plasma RNA was gradually decreased in patients with an increased number of mutations listed in this set (fosamprenavir/ritonavir mutation score A). When none to three mutations were present, the median HIV-1 plasma RNA decrease was \(-1.64 \log_{10} \text{copies/mL}\), whereas it was \(-0.11\) \log_{10} \text{copies/mL} in patients with four to six mutations. We thus considered as non-resistant the group of isolates with 0–3 mutations and as resistant the group with more than 3 mutations (Figure 2b).

In order to evaluate the possibility of constructing a mutation score taking into account both favourable and unfavourable mutations, we repeated the analysis by starting from the 12 abovementioned mutations associated with a worse VR, plus the two mutations (V77I and N88S) associated with a better VR. This led us to select a new score (score B: L10FIV + L33F + M36I + I54L/M/V/A/T/S + I62V + V82A/F/C/G + I84V and L90M) also highly associated with VR (Jonckheere–Terpstra test, \(P = 1.94 \times 10^{-7}\)). When the score B was between 1 and 3 (n = 47 patients), the median VR was \(-1.50 \log_{10} \text{copies/mL}\), whereas the median VR was \(-0.01 \log_{10} \text{copies/mL}\) in patients with a score between 4 and 6 (n = 26 patients).

Multivariate analysis

In a preliminary univariate analysis, a fosamprenavir/ritonavir mutation score A \(>3\), a lower RTI-GSS, co-administration of
nevirapine or tenofovir with fosamprenavir/ritonavir and previous exposure to lopinavir or amprenavir were associated with a worse VR. In contrast, co-administration of enfuvirtide and the presence of the protease mutations N88S and V77I were associated with a better VR. The multivariate analysis (Figure 3) identified a fosamprenavir/ritonavir mutation score A \( > 3 \) (\( P < 0.001 \)) and co-administration of tenofovir (\( P < 0.004 \)) as independent predictors of a worse VR, whereas co-administration of enfuvirtide in enfuvirtide-naive patients (\( P < 0.002 \)) and the presence of the protease mutation N88S (\( P < 0.001 \)) remained associated with a better VR. We investigated if co-administration of didanosine and tenofovir could explain the association of tenofovir with a worse VR; didanosine and tenofovir were co-administered in only four patients. For these four patients, the median VR was \( +0.31 \log_{10} \text{copies/mL} \) versus \( -0.43 \log_{10} \text{copies/mL} \) in patients with tenofovir but no didanosine and \( -1.55 \log_{10} \text{copies/mL} \) in other patients (\( P = 0.001 \)).

A multivariate analysis taking into account fosamprenavir/ritonavir mutation score B showed that a score B \( > 3 \) (\( P < 0.001 \)) and co-administration of tenofovir (\( P = 0.011 \)) were independently associated with a worse VR, whereas co-administration of enfuvirtide in enfuvirtide-naive patients (\( P < 0.002 \)) remained associated with a better VR.

**Validation of a new algorithm for fosamprenavir/ritonavir**

The fosamprenavir/ritonavir mutation score A was included in the 2006 version of the ANRS interpretation algorithm (http://www.hivfrenchresistance.org), in which resistance to fosamprenavir/ritonavir is defined by (i) the 150 mutation, (ii) the presence of the mutations V32I and I47V/A; or (iii) a fosamprenavir/ritonavir mutation score A \( > 3 \). We compared this new algorithm with previously proposed algorithms (Table 3), showing that it was highly associated with the VR, in contrast with the previous version of the ANRS algorithm, and led to the highest discrimination in VR for the susceptible versus resistant groups.

**Fosamprenavir plasma levels**

Overall, 46 patients had a fosamprenavir plasma determination, either at month 1 or at month 3, with a median fosamprenavir plasma level of 2305 ng/mL (IQR: 1729–3992). There was no difference in the median fosamprenavir plasma levels between patients with and without co-administration of tenofovir with fosamprenavir/ritonavir (\( P = 0.384 \)).

**Discussion**

In this population of PI-experienced patients receiving fosamprenavir/ritonavir in the context of salvage therapy, we defined two
different sets of PI resistance mutations associated with VR. Fosamprenavir/ritonavir score A took into account only mutations associated with a worse VR, leading to the score L10F/I/V + L33F + M36I + I54L/M/V/A/T/S + I62V + V82A/F/C/G + I84V + L90M. Within these eight mutations, two (54 and 84) had been reported to be selected in vitro or in vivo by amprenavir. The other mutations too have been already listed by the IAS-USA panel. The substitutions at codons 33, 82, 84 and 90 are considered as major PI resistance mutations and are involved in multiple PI cross-resistance patterns. This set of eight mutations has been shown to be highly associated with VR by using a stepwise methodology already applied for defining interpretation algorithms for other antiretroviral compounds.\(^{10–12}\) We have shown that a number of \(>3\) mutations within the fosamprenavir/ritonavir score was associated with a significant decrease of viral load response and thus
could be considered to predict resistance to fosamprenavir/ritonavir. We conducted a multivariate analysis of the factors associated with VR showing that prior genotypic resistance to fosamprenavir/ritonavir using the newly defined score was independently associated with a poorer VR. Interestingly, the presence of N88S mutation at baseline was associated with a better VR in this multivariate analysis. This finding is in accordance with previous reports suggesting that this mutation could be responsible for *in vitro* hypersusceptibility to amprenavir and associated with a better VR to this drug. Co-prescription of enfuvirtide was also associated with a better VR as already shown in all recent salvage trials of ART. Tenofovir use was a predictor of a worse VR in this highly ART-experienced population. In order to explain this finding, we examined the fosamprenavir plasma levels between patients receiving or not receiving tenofovir, but no difference could be found from our data. It is noteworthy that four patients receiving didanosine/tenofovir had a worse VR than the other patients with tenofovir and no didanosine; this could at least partly explain the association between tenofovir and a poor VR, since the didanosine/tenofovir combination has been shown to lead to suboptimal virological and immunological responses.

In order to take into account both mutations associated with a worse or with a better VR, we built the fosamprenavir/ritonavir score B: L10FIV + L33F + M36I + I54L/M/V/A/T/S + A71V – V77I – N88S + L90M, which had the highest association with the virological outcome. Fosamprenavir/ritonavir score B remained associated with VR in a multivariate analysis. We, however, selected fosamprenavir/ritonavir score A for inclusion into the ANRS interpretation algorithm for fosamprenavir/ritonavir, since fosamprenavir/ritonavir score B did not retain two primary PI resistance mutations from score A (V82A/F/G/C/I + I84V) and thus was likely to overestimate the genotypic sensitivity to fosamprenavir/ritonavir, as shown by the smaller number of patients with isolates considered as resistant to fosamprenavir/ritonavir with score B compared with score A.

Some substitutions previously associated with resistance to amprenavir/ritonavir could not be accounted for in the fosamprenavir/ritonavir resistance scores because of their low prevalence in the studied population. Indeed, these mutations (I50V, V32I and I47V) are more frequently observed in first-line failures on unboosted fosamprenavir (and have rare occurrence with fosamprenavir/ritonavir) rather than in multiple PI-experienced patients. However, we have included these mutations in the new ANRS interpretation algorithm for fosamprenavir/ritonavir, along with the fosamprenavir/ritonavir mutation score A.

Compared with previously published algorithms, our new algorithm was shown in this study to be both highly predictive and discriminant with regard to VR. Two other algorithms based on similar lists of mutations were also shown to be predictive. By contrast, the former version of the ANRS algorithm 2005, which was based on a study of patients receiving amprenavir/ritonavir, was not predictive of VR in our patients, justifying its replacement by the newly validated algorithm.

One difficulty in the design of genotypic scores is that they depend of the representation of the different resistance mutations and/or polymorphisms in the studied viral population. Some limitations of our analysis cannot be circumvented: the limited number of patients and the limited genetic diversity with a great majority of HIV-1 B viruses could have influenced the results. Moreover, as for most patients, fosamprenavir/ritonavir was chosen based on genotypic resistance testing results; this could be one reason for the limited capability of the data set to detect the role of previously recognized mutational patterns.

We explored the role of all the represented protease substitutions including polymorphic residues not listed by the IAS group; two of them (E34K/G/Q and I72K/L/R/V) were associated with a poorer response in the univariate analysis but did not significantly improve the predictivity of the score. Furthermore, these polymorphisms were highly associated with the presence of the L10I mutation, which was kept in the score (data not shown). Finally, a recent study based on a population of patients enrolled into the fosamprenavir/ritonavir CONTEXT and TRIAD trials, as well as a study from a Spanish group, enabled an external validation of the fosamprenavir/ritonavir

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**Table 3.** Comparison of four interpretation algorithms for resistance to fosamprenavir/ritonavir

<table>
<thead>
<tr>
<th>Fosamprenavir/ritonavir algorithm</th>
<th>Resistance level</th>
<th>n</th>
<th>Mean change in VL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANRS 2005</td>
<td>sensitive</td>
<td>70</td>
<td>−1.07</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>resistance</td>
<td>3</td>
<td>−0.93</td>
<td>0.62</td>
</tr>
<tr>
<td>Stanford 1.4.4</td>
<td>sensitive</td>
<td>19</td>
<td>−1.98</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>possible</td>
<td>30</td>
<td>−0.94</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>resistance</td>
<td>24</td>
<td>−0.50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Zephir study</td>
<td>sensitive</td>
<td>26</td>
<td>−1.82</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>resistance</td>
<td>47</td>
<td>−0.65</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ANRS 2006</td>
<td>sensitive</td>
<td>33</td>
<td>−1.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>resistance</td>
<td>40</td>
<td>−0.39</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

ANRS 2005 and 2006 algorithms are available at [http://www.hivfrenchresistance.org](http://www.hivfrenchresistance.org), the Stanford algorithm is available at [http://hivdb.stanford.edu](http://hivdb.stanford.edu) and the Zephir study is referenced in Pellegrin et al. Mean change in VL: mean change in plasma HIV-1 RNA (*log*₁₀ copies/mL) between baseline and month 3 on fosamprenavir/ritonavir.
score A in these independent data sets. These findings confirm the robustness of this score and warrant its interest for the management of ART in PI-experienced patients.

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Transparency declarations

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References


