ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 2004, p. 4687–4692 Vol. 48, No. 12
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.12.4687–4692.2004

Clinically Relevant Interpretation of Genotype and Relationship to Plasma Drug Concentrations for Resistance to Saquinavir-Ritonavir in Human Immunodeficiency Virus Type 1 Protease Inhibitor-Experienced Patients

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Received 23 January 2004/Returned for modification 13 May 2004/Accepted 17 August 2004

It has been shown that virological protease inhibitor (PI) resistance mutations present at the initiation of saquinavir (SQV) plus ritonavir (RTV) therapy in PI-experienced patients are the strongest predictors of virological response. But most of the current resistance algorithms are adapted for unboosted SQV regimens. We applied a stepwise methodology for the development and validation of a clinically relevant genotypic resistance score for an SQV (800 mg twice per day [b.i.d.]) plus RTV (100 mg b.i.d.)-containing regimen. PI-experienced patients treated by this regimen achieved a human immunodeficiency virus plasma viral load (VL) of <200 copies/ml at months 3 to 5 for 41.7% of subjects. Adjusted in a multivariate analysis, taking into account all the confounding factors, such as the nucleoside used, five mutations were combined in a resistance score associated with a reduced virological response to an SQV-plus-RTV regimen: L24I, I62V, V82A/F/T/S, I84V, and L90IM. Patients with isolates harboring 0 to 1 mutation among the score achieved -2.20 $\log_{10}$ and -1.23 $\log_{10}$ copies/ml of VL reduction, respectively, while it was -0.27 $\log_{10}$ copies/ml for those with at least two mutations, classifying the isolates as "no evidence of resistance" (0 or 1 mutation) or "resistance" (≥2 mutations). The minimum concentration in plasma (C_{min}) of SQV alone was not associated with the virological response. However, the combination of the SQV C_{min} and the genotypic score, expressed as the genotypic inhibitory quotient, was predictive of the virological response, suggesting that the interpretation of SQV concentrations in plasma should be done only in the context of the resistance index provided by viral genotype for PI-experienced patients.

Saquinavir (SQV) is a potent protease inhibitor (PI) in vitro, but its clinical activity when used as a single PI is hampered by limited oral bioavailability (3, 6). Ritonavir (RTV) is a potent inhibitor of cytochrome P450 isoenzymes (CYP 3A4 and 2D6), and when it is coadministered with SQV, an approximately 20-fold increase in SQV plasma exposure is achieved (9). Consequently, the coadministration of SQV with a low dose of RTV (100 mg twice per day [b.i.d.]) increases the exposure to SQV without having a substantial impact on tolerability. A number of clinical trials have now evaluated SQV-plus-RTV (SQV/r) treatment regimens, at dosages of 1,000 mg/100 mg b.i.d. and 1,600 mg/100 mg once daily, showing a potent and sustained viral suppression in PI-naive and -experienced patients (17). The increase of the minimum concentration in plasma (C_{min}) of SQV obtained by the addition of RTV should have the potential to overcome PI resistance that is crucial in salvage therapy, and this combination, with concurrent nucleoside reverse transcriptase inhibitor (NRTI) therapy, has been described as a possible salvage regimen after failure of indinavir, ritonavir, or nelfinavir therapy.

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Reduced susceptibility to SQV is most often associated with acquisition of the G48V and L90M mutations in human immunodeficiency virus type 1 (HIV-1) protease when SQV is used as the first PI (10, 11). Single mutations result in approximately 10-fold changes, with the less-frequent double mutations leading to reductions in sensitivity of up to 100-fold (5, 12). Additional mutations at codons 10, 36, 63, 71, 73, 82, and 84 have also been reported to arise during SQV therapy (4, 10, 11, 18). The most common mutation selected by SQV, L90M, confers cross-resistance to other PI, especially when associated with minor mutations. The specific mutation G48V is selected later than the L90M mutation and at higher SQV concentrations (22).

It has been shown that PI mutations present at the initiation of SQV/r therapy for PI-experienced patients were the strongest predictors of virological response (23). But most of the current resistance algorithms take into account the mutations impacting the virological response to unboosted SQV therapy. Since it is now widely recognized that correlation studies analyzing the virological response in treatment-experienced patients according to the viral genotypic profile at baseline provide relevant information for establishing resistance algorithms, we developed a clinically relevant viral genotype interpretation for resistance to SQV/r. Moreover, several studies have shown the usefulness for some boosted PIs of com-

TABLE 1. Baseline patient characteristics[a]

Parameter	Value
No. (%) at CDC stage C[a]	15 (22%)
Mean plasma HIV-1 RNA log copies/ml (SD)	4.38 (0.76)
Mean CD4 cell count/mm^3 (SD)	292 (187)
Mean duration [mo (SD)] of exposure to:	
Antiretroviral agents	46 (36)
Protease inhibitors	24 (15)
Median no. (range) of previous:	
Antiretroviral agents	5 (3–8)
Nucleoside analogues	3 (2–6)
No. (%) of pts treated with:	
1 protease inhibitor	51 (70.8)
2 protease inhibitors	21 (29.2)
At least 1 nonnucleoside analog	16 (22)
No. (%) of pts with protease inhibitor exposure	
Indinavir	49 (68)
Nelfinavir	19 (26)
Ritonavir	13 (18)
Nonboosted saquinavir	10 (14)
Boosted indinavir	8 (11)
No. (%) of pts treated with NRTI associated with SQV/p	
3TC	51 (70.8)
d4T	50 (69.4)
ddI	28 (38.9)
ABC	10 (13.9)
ZDV	9 (12.5)
HU	1 (1.4)

[a] CDC stage C, AIDS status according to the Centers for Disease Control and Prevention classification.

[b] SD, standard deviation; pts, patients; NRTIs, nucleoside reverse transcriptase inhibitors; SQV/r, saquinavir boosted by ritonavir; 3TC, lamivudine; d4T, stavudine; ddI, didanosine; ABC, abacavir; ZDV, zidovudine; HU, hydroxyurea.

bining plasma PI concentrations with genotypic score, expressed as genotypic inhibitory quotient (GIQ), to enhance the predictivity of the virological response, and we analyzed this parameter in this study (7, 14, 15, 20).

(This work was presented previously during the XII International HIV Drug Resistance Workshop, Los Cabos, Mexico, 10 to 14 June 2003 [abstr. 107], and during the 2nd International AIDS Society (IAS) Conference on HIV Pathogenesis and Treatment, Paris, France, 13 to 16 July 2003 [abstr. 826].)

MATERIALS AND METHODS

Patients and antiretroviral regimens. Seventy-two PI-experienced patients were retrospectively selected. They were treated at baseline with two or three NRTIs and RTV (100 mg b.i.d.) plus SQV (800 mg b.i.d.). No nonnucleoside reverse transcriptase inhibitors or PIs other than SQV and RTV were used in the antiretroviral combinations. The characteristics of patients at baseline are presented in Table 1.

HIV-1 RNA quantification. Quantification of HIV-1 RNA in plasma was performed at baseline and after 3 to 5 months of treatment using the Amplicor Monitor assay (Cobas 1.5 test; Roche Diagnostics, Bazel, Switzerland) with a detection limit of 200 copies/ml.

Genotypic resistance testing. Plasma samples for determination of viral genotype were collected at baseline. The reverse transcriptase and protease gene sequences were determined using population sequencing according to the Agence Nationale de Recherches sur le SIDA consensus method with an ABI 3100 genetic analyzer (PE Applied Biosystems) (1). The sequences were analyzed by using Sequence Navigator software (PE Applied Biosystems) and reported as amino acid changes with respect to the sequence of the wild-type virus HXB2.

Determinations of PI concentrations in plasma. Plasma samples were collected in heparinized tubes approximately 12 h after the last intake at steady state at months 3 to 5. SQV plasma concentrations were measured by a specific and validated high-performance liquid chromatographic assay coupled with UV detection at 240 nm. Briefly, SQV and its internal standard were isolated from plasma after alkalinization by a double-step back liquid-liquid extraction by *tert*-methylbutylether. The separation was achieved on an octadecylsilyl analytical column with a mobile phase containing 0.1% trifluoroacetic acid, acetonitrile, and methanol (55:45 [vol/vol]). The assay was found to be linear and has been validated over the concentration range of 9 to 1,000 μg/liter for SQV from 500 μl of plasma. The coefficients of variation within a day and between days were 4.6 and 4.5%, respectively. The lower limit of quantification was 9 ng/ml and was defined as the concentration for which the relative standard deviation and the percent deviation from the nominal concentration were lower than 20%.

On the same samples, the RTV C_{min} was determined by a high-performance liquid chromatographic assay coupled with UV detection after liquid-liquid phase extraction as described previously (16). The RTV method was validated over a plasma concentration range of 30 to 15,000 ng/ml with a quantification limit of 30 ng/ml. SQV and RTV were kindly provided by Roche, Inc., and Abbott, Inc., respectively.

The GIQ was calculated as a ratio of steady-state SQV C_{min} in plasma at months 3 to 5 to number of baseline specific SQV protease mutations with the final genotypic score (set 1).

Statistical methods. The end-point for the analysis was the change in plasma HIV-1 RNA (log copies/ml) between day 0 and months 3 to 5. First we analyzed the impact of the presence of each mutation along the protease gene (codons 1 to 99) on the virological response by comparing the change in plasma HIV-1 RNA in patients with and without the mutation using a nonparametric Mann-Whitney test. Mutations which were present in at least 5% of patients and for which the *P* value was lower than 0.10 in the above univariate analysis were retained and then analyzed in a backward multivariate linear regression in order to select the mutations with a *P* value of <0.10. We calculated the average response for patients harboring viruses with an incremental number of mutations (0, 1, 2, etc.) among those which were retained in the backward regression and studied the association between this number of mutations and the change in plasma HIV-1 RNA using a nonparametric Kruskal-Wallis (KW) test. We also tested whether adding the mutations that were not retained by the backward regression and adding the mutations described in the IAS list would improve the *P* value and should therefore be kept in the score. Taking into account the results observed, two levels of resistance were defined, depending on the number of mutations, representing the genotypic score: resistance and no evidence of resistance.

Then, to assess whether or not the genotypic score was an independent predictor of response, a linear multivariate regression was used, accounting for the baseline variables which were predictive of response in the univariate analysis ($P < 0.10$).

Finally we used the bootstrap resampling method to assess the robustness of the score obtained. This approach was initially suggested for cross-validation of the Cox regression model (19). Univariate and multivariate analyses of the score were performed on 100 samples drawn from the initial 72 patients by sampling with replacement. For the univariate analyses, we report the mean changes in viral load and the number of times the score had a *P* value below 0.001 in the resistance groups, while for the multivariate analyses, we report the mean value of the regression parameter and its standard deviation; we also report the numbers of times the score had *P* values below 0.05 and below 0.10.

For the pharmacological results, correlations were calculated with the Spearman correlation coefficient.

The statistical program used for analyses was SPSS (version 11.5) for Windows (SPSS, Inc.).

RESULTS

Baseline patient characteristics. All patients included were monitored in a single medical center (Pitié-Salpêtrière Hospital, Paris, France), and all of them had available baseline viral genotype and viral load measurements at baseline and months 3 to 5. The details of the antiretroviral drugs associated with RTV plus SQV are summarized in Table 1. For the 72 patients, the median numbers of major and minor PI mutations among

TABLE 2. Univariate analysis of virological response according to presence of mutated or wild-type codons at specific sites of the protease gene[a]

Site and WT[a] amino acid	Product(s) of WT or mutated codon	No. of isolates with codon	Mean decrease in VL (SD)	*P* value (Mann-Whitney)
L10	L	40	−1.5510 (1.19754)	0.003
	I, R, V, F	32	−0.6859 (0.96078)	
L24	L	66	−1.2389 (1.19091)	0.080
	I	6	−0.3700 (0.54966)	
M46	M	47	−1.4393 (1.23546)	0.018
	I, L	25	−0.6537 (0.85555)	
G48	G	67	−1.2332 (1.17802)	0.055
	V	5	−0.2730 (0.70180)	
I54	I	56	−1.2920 (1.18422)	0.081
	V	16	−0.7272 (1.05669)	
I62	I	52	−1.3745 (1.16537)	0.015
	V	20	−0.6258 (1.03855)	
A71	A	43	−1.4158 (1.25267)	0.033
	T, V	29	−0.7970 (0.95081)	
V82	V	41	−1.5429 (1.24076)	0.003
	A, F, S, T	31	−0.6687 (0.87138)	
I84	I	61	−1.3764 (1.14977)	<0.001
	V	11	−0.0028 (0.30815)	
L90	L	53	−1.4675 (1.16881)	0.001
	I, M	19	−0.3271 (0.69214)	
T12	T	63	−1.0211 (1.10030)	0.010
	A, D, N, P, Q, S	9	−2.1843 (1.23155)	
I13	I	52	−0.9645 (1.10941)	0.038
	V	20	−1.6916 (1.20185)	

[a] WT, wild-type.

the IAS-USA panel (http://www.iasusa.org) were 1 (0 to 4) and 3 (0 to 8), respectively. For these patients the most frequent nucleoside analogues coprescribed with SQV/r were lamivudine, in 51 patients (70.8%), and stavudine, in 50 patients (69.4%) (Table 1).

Virological response to r/SQV-containing regimen. The mean decrease in plasma HIV RNA between baseline and months 3 to 5 in the patients exposed to SQV/r was 1.17 ± 1.17 $\log_{10}$ copies/ml (mean ± standard deviation). For 30 patients (41.7%), HIV RNA in plasma was below 200 copies/ml at months 3 to 5.

Impact of the PI mutations on the virological response. Ten mutations were found to be associated with a reduced virological response to SQV/r ($P < 0.1$): L10F/I/R/V, L24I, M46I/L, G48V, I54V, I62V, A71T/V, V82A/F/S/T, I84V, and L90IM. Table 2 shows the univariate analysis of the virological response according to the presence of mutated or wild-type codons at specific sites of the protease gene associated with resistance to PIs. Mutations at codons 10, 46, 62, 71, 82, 84, and 90 were significantly associated with a reduced virological response ($P < 0.05$), and mutations at codons 24, 48, and 54 were also retained for further analyses ($P < 0.1$). Two mutations were significantly associated with a better virological response to SQV/r ($P < 0.05$): T12A/D/N/P/Q/S and I13V.

Boosted SQV/r genotypic score. (i) Univariate analysis. From the univariate analysis we retained 10 PI mutations, at codons 10, 24, 46, 48, 54, 62, 71, 82, 84, and 90, and in the multivariate analysis 5 mutations were retained: 4 mutations at codons 62, 82, 84, and 90 remained significant ($P < 0.005$), and a mutation at codon 24 was also retained ($P < 0.10$). Table 3 shows the chi-square values and the *P* values of the KW analysis of the mean decrease in viral load according to the number of substitutions for different combinations of mutations. Based on the chi-square and *P* values, three combinations of mutations showed a strong association between the decrease in viral load response and the number of mutations observed when using these sets, including not only the PI mutations among the IAS list but also some other PI mutations that were predictive of virological response in the univariate analysis.

The I62V mutation was not previously described as a protease mutation able to decrease the virological efficacy of a PI.

TABLE 3. Combinations of mutations[a]

Combination of mutations	Chi square	*P* value[b]
Set 1: L24I, I62V, 82AFTS, I84V, L90IM	33.5	**2.51×10^{-7}**
Set 1 + L10IRVF	30.1	4.69×10^{-6}
Set 1 + M46I	29.1	7.55×10^{-6}
Set 1 + G48V	35.4	**3.88×10^{-7}**
Set 1 + I54V	30.1	4.62×10^{-6}
Set 1 + A71VT	29.4	6.62×10^{-6}
Set 1 + G48V + L10IRVF	32.7	4.26×10^{-6}
Set 1 + G48V + M46I	30.5	1.15×10^{-5}
Set 1 + G48V + I54V	30.6	3.73×10^{-6}
Set 1 + G48V + A71VT	30.5	1.18×10^{-5}
Set 1 + G48V + V77I	25.5	4.00×10^{-5}
Set 1 + G48V + G73S	35.9	**3.04×10^{-7}**
Set 1 + G48V + G73S + V77I	26.2	8.00×10^{-5}

[a] *P* value of the univariate analysis (Kruskal-Wallis) assessing the mean decrease in viral load according to the number of mutations in each different set.
[b] Boldface indicates a combination of mutations assessing the strongest association with the decrease in viral load response.

TABLE 4. Association of I62V protease mutation with other PI resistance mutations according to the IAS list

IAS mutation(s) associated with I62V (n = 20)	No. (%) of patients[a]
L10IRVF	11 (55)
L24I	1 (5)
M46IL	10 (50)
G48V	3 (15)
I54V	5 (25)
A71VT	8 (40)
G73S	4 (20)
V77I	10 (50)
V82AFTS	11 (55)
I84V	4 (20)
L90IM	7 (35)

[a] No. of patients with isolate harboring mutation.

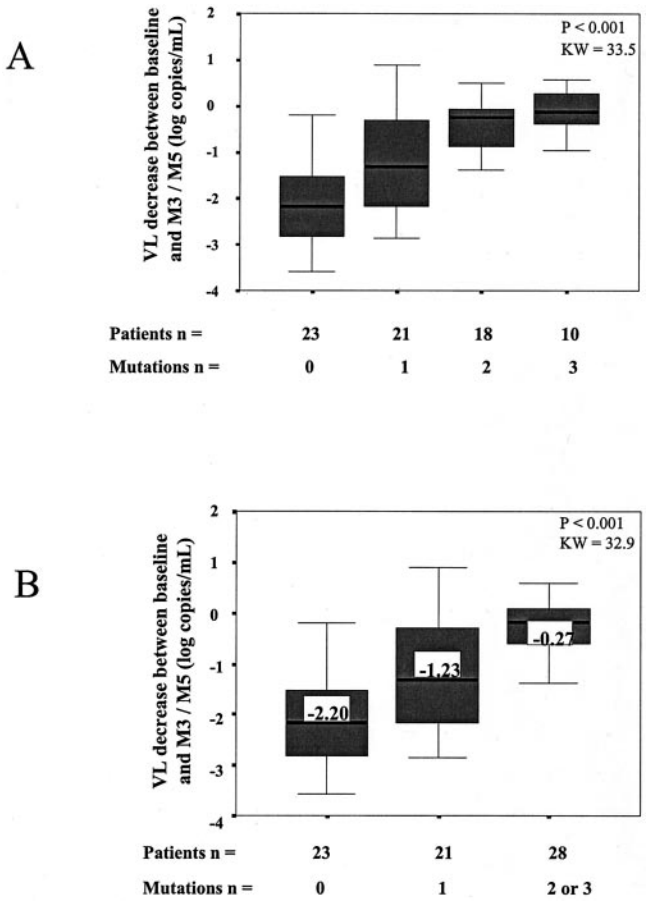

FIG. 1. Median decrease in VL, categorized by number of mutations present among L24I, I62V, V82AFTS, I84V, and L90IM. M3, month 3; M5, month 5.

In this study, this mutation was present in isolates from 20 patients, and its association with other PI resistance mutations is presented in Table 4. Moreover, when the I62V mutation was present, in most of the cases (all but 3 out of the 20 patients) it was associated with at least one of the PI resistance mutations within the IAS list.

(ii) Multivariate and bootstrap analyses. Eight variables were predictive of response in the univariate analysis ($P < 0.10$): six variables were associated with a decrease of the virological response (baseline $\log_{10}$ viral load [VL], previous prescription for at least four NRTIs, previous prescription of nonboosted SQV, prescription of stavudine at baseline, reverse transcriptase mutations K70R and M184VI at baseline); two variables were associated with an increase of the virological response (protease mutations T12A/D/N/P/Q/S and I13V at baseline). In the multivariate analysis, taking into account all the predictive variables, the SQV/r genotypic score (set 1, L24I, I62V, 82AFTS, I84V, and L90IM) was the best independent predictor of the virological response at months 3 to 5, compared to the other two combinations of mutations (1 mutation versus 0 mutations, $P = 0.007$; at least 2 mutations versus 0 mutations, $P < 0.001$). Among the variables tested in the multivariate analysis, only the SQV/r mutation score, the previous use of SQV, and the baseline viral load remained significantly associated with the virological response.

In the bootstrap analysis, set 1 was also the strongest predictor of the virological response: in the 100 bootstrap samples, the mean decreases for the univariate analysis in plasma HIV-1 RNA were −2.22, −1.25, and −0.28 in patients with zero, one, or at least two mutations, respectively. The P value was significant ($P < 0.001$) in 100 cases. For the multivariate analyses, the mean β parameter and the standard deviation of the score were estimated to be 0.70 ± 0.25 for one mutation versus no mutations and 1.2 ± 0.3 for at least two mutations versus no mutations. The P value was significant (<0.05) in 77 cases and lower than 0.10 in 86 cases for one mutation versus none, and the P value was significant (<0.05) in 98 cases and lower than 0.10 in 99 cases for at least two mutations versus none.

Figure 1A shows the mean decrease in viral load according to number of mutations from the best combination (L24I, I62V, 82AFTS, I84V, and L90IM) ($P < 0.001$; KW value = 33.5). The decrease in viral load was significantly less when the number of mutations increased. To build the resistance score, we grouped the numbers of mutations for which the viral load reductions were similar. When no mutation was present at baseline, the mean reduction in plasma HIV-1 RNA was −2.20 $\log_{10}$ copies/ml, while it was −1.23 and −0.27 $\log_{10}$ copies/ml in patients with one or at least two mutations, respectively (Fig. 1B) ($P < 0.001$; KW value = 32.9). We therefore defined viral isolates as "not resistant" and "resistant" when they possessed fewer than two mutations or at least two mutations, respectively.

Pharmacological and GIQ results. Median SQV and RTV plasma C_{min} values measured at months 3 to 5 were 328 ng/ml (9 to 5,400 ng/ml) and 480 ng/ml (30 to 4,493 ng/ml), respectively. A wide interindividual variability of C_{min} in plasma was found for SQV and RTV. The interval of the last drug intake and sampling recorded was voluntarily reported by patients.

There was a correlation between SQV and RTV C_{min} values measured at month 4 ($r = 0.712$; $P < 0.001$), but there was no correlation between the virological response either with SQV C_{min} ($r = -0.191$; $P = 0.119$) or RTV C_{min} ($r = -0.038$; $P = 0.761$). However, there was a correlation between the decrease of plasma HIV-1 RNA between day 0 and months 3 to 5 and the GIQ ($r = -0.402$; $P = 0.006$).

DISCUSSION

HIV-1 genotypic resistance testing provides prognostic information for patients who are experiencing a less-than-optimal virologic response to antiretroviral therapy. Correlation studies analyzing the virological response in treatment-experienced patients according to the genotypic profile at baseline should provide the most relevant information for establishing algorithms (13). A stepwise methodology for the development and validation of clinically relevant genotypic resistance scores for antiretroviral drugs was previously proposed (2). Here we applied this method to an SQV-plus-RTV-containing regimen with PI-experienced patients.

SQV was primarily used in combination with RTV, because SQV has a low and variable bioavailability, resulting in low concentrations in plasma and subsequent evolution of viral resistance (8). A commonly used combination is SQV plus RTV at 1,000 mg and 100 mg, respectively, twice a day, which results in adequate SQV concentrations in plasma (21). In our study, PI-experienced patients were treated with SQV plus RTV at 800 and 100 mg b.i.d., respectively, and achieved a VL of <200 copies/ml at months 3 to 5 in 41.7% of subjects. This can be compared to the results of the MaxCmin1 study where 60% of subjects achieved maximum virological control with 39% of naive patients (17).

In our study, five mutations were combined in a resistance score associated with a reduced virological response to SQV/r: L24I, I62V, V82A/F/T/S, I84V, and L90IM. Patients with isolates harboring zero to one of these SQV/r resistance mutations achieved -2.20 $\log_{10}$ copies/ml and -1.23 $\log_{10}$ copies/ml of VL reduction, respectively, while it was -0.27 $\log_{10}$ copies/ml in those with at least two mutations. The genotypic score classifies the isolates as showing no evidence of resistance (0 to 1) or resistance (≥2) according to the number of mutations. This result is not very different from that observed in a previous study showing a significant reduction of the virological response to SQV/r when isolates harbored any two mutations among positions 30, 46, 48, 54, 82, 84, and 90 (23). The different subset of mutations identified between the two studies may be due in part to the different amounts of ritonavir and SQV given to patients (300 to 400 mg of ritonavir plus 400 to 600 mg of SQV b.i.d. versus 100 mg of ritonavir plus 800 mg of SQV b.i.d.). The genotypic score determined in our study is adapted for the use of SQV/r (800/100 mg b.i.d.), but it does not mean that it will be relevant for other amounts of ritonavir and SQV given to patients. Previous rules for HIV-1 genotypic resistance testing recommended ruling out the use of SQV when only the mutation G48V, I84V, or L90M was present. However, these rules were based mainly on unboosted SQV-containing regimen data. The I62V mutation was identified in this data set to be involved in the reduction of the SQV/r virological efficacy. This mutation was not previously described as a protease mutation that can decrease PI efficacy. But most previous studies did not analyze all the amino acid positions in the protease gene, and this could explain the fact that new mutations, such as I62V, could be identified, using this approach to determine PI cross-resistance mutations. This study also showed that in most cases, the I62V mutation was associated with at least one of the known PI resistance mutations, strongly suggesting its role in PI cross-resistance.

The increase of plasma SQV levels with the use of RTV, as confirmed by a median of SQV C_{min} at 328 ng/ml, explains the differences between the mutation sets, since it is well known that this can overcome resistance, in some cases increasing the PI clinical cutoff.

The pill count for an SQV/r regimen (800/100 mg b.i.d.), using the 200-mg SQV galenic formulation, is 10 pills per day (4 SQV capsules plus 1 RTV capsule, b.i.d.). A new formulation, the 500-mg SQV mesylate tablet, is under development and will allow the use of only two SQV tablets twice daily for an SQV/r regimen (1,000/100 mg b.i.d.). The lower pill count might improve adherence to SQV/r regimens. This new formulation might allow increasing the SQV exposure for PI-experienced patients, in order to overcome PI resistance, with a low number of pills, especially since no upper limit of the therapeutic range of SQV C_{min} in plasma has been identified to date, and consequently, this potential increased efficacy should not be to the detriment of the patient's tolerance. This should be further validated with PI-experienced patients receiving the 500-mg SQV tablets. Moreover, strategies based on a double-boosted PI regimen in order to enhance simultaneously the drug levels of two PIs (i.e., saquinavir/lopinavir/ritonavir, 1,000/400/100 mg b.i.d.) have been tested successfully in several clinical trials with highly treatment-experienced patients. Combinations of potent PIs that lack cross-resistance and with favorable pharmacokinetic profiles should have the potential to be active on variants with different patterns of resistance.

This study confirms, as previously described, the relationship between C_{min} of SQV and RTV when a low dose of RTV is used (C. Lamotte, M. Lafay, J. Reynes, J. L. Vildé, P. Yéni, C. Katlama, and G. Peytavin, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. A-1613, 2003). Interestingly, the C_{min} of SQV alone is not associated with virological response. Measurement of SQV in plasma could have been done too late in this study, thus explaining the absence of correlation between the C_{min} value of SQV and the virological response. It might have been more relevant to measure the plasma levels much earlier after treatment change (14). The combination of SQV C_{min} and genotypic score expressed as GIQ was predictive of the virological response but does not enhance the predictivity comparing to the genotypic score used alone. These results suggest that the interpretation of plasma SQV concentrations should be done only in the context of the resistance index provided by viral genotype for PI-experienced patients. The development of such algorithms, combining virological mutations and PI trough levels, should be validated in prospective clinical trials.

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