Human Immunodeficiency Virus Type 1 Genotypic and Pharmacokinetic Determinants of the Virological Response to Lopinavir-Ritonavir-Containing Therapy in Protease Inhibitor-Experienced Patients

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The response to regimens including lopinavir-ritonavir (LPV/r) in patients who have received multiple protease (PR) inhibitors (PI) can be analyzed in terms of human immunodeficiency virus type 1 (HIV-1) genotypic and pharmacokinetic (pK) determinants. We studied these factors and the evolution of HIV-1 resistance in response to LPV/r in a prospective study of patients receiving LPV/r under a temporary authorization in Bordeaux, France. HIV-1 PR and reverse transcriptase sequences were determined at baseline LPV/r for all the patients and at month 3 (M3) and M6 in the absence of response to treatment. pK measurements were determined at M1 and M3. Virological failure (VF) was defined as a plasma viral load ≥ 400 copies/ml at M3. A multivariate analysis of the predictors of VF, including clinical and biological characteristics and the treatment history of the patients, was performed. The PR gene sequence at M0, including individual mutations or a previously defined LPV mutation score (D. J. Kempf, J. D. Isaacson, M. S. King, S. C. Brun, Y. Xu, K. Real, B. M. Bernstein, A. J. Japour, E. Sun, and R. A. Rode, *J. Virol.* 75:7262–7269, 2001), and the individual exposure to LPV were also included covariates. Sixty-eight patients were enrolled. Thirty-four percent had a virological response at M3. An LPV mutation score of >5 mutations, the presence of the PR I54V mutation at baseline, a high number of previous PIs, prior therapy with ritonavir or indinavir, absence of coprescription of efavirenz, and a lower exposure to LPV or lower LPV trough concentrations were independently associated with VF on LPV/r. Additional PI resistance mutations, including primary mutation I50V, could be selected in patients failing on LPV/r. Genotypic and pK parameters should be used to optimize the virological response to LPV/r in PI-experienced patients and to avoid further viral evolution.

Treatment of human immunodeficiency virus (HIV)-infected individuals with combination therapy including protease inhibitors (PI) results in a significant suppression of HIV replication (1, 2, 3, 11) and in improvement in clinical outcomes, with marked reductions in HIV-associated morbidity and mortality (5, 9). However, the efficacy of antiretroviral (ARV) treatment can be impaired by several factors, including poor

compliance with treatment regimens, suboptimal antiviral potency and drug concentrations, and selection of ARV-resistant HIV quasiespecies (6). Resistance to PI is driven by the selection of primary mutations located close to the active site of the HIV type 1 (HIV-1) protease, producing significant changes in the affinity of the binding of the inhibitor to the mutant active site (4) and often occurs early during virological rebound. Secondary resistance mutations may be selected later and may compensate for the initial decrease of viral fitness related to the appearance of primary mutations. These secondary mutations tend to be common to all PI, facilitating the emergence of resistance to the whole PI class.

Lopinavir (LPV)-ritonavir (LPV/r) is a coformulation of lopinavir, an HIV PI, and low-dose ritonavir, which inhibits LPV metabolism and which enhances plasma LPV levels (12). LPV/r has shown significant potency in treatment-naïve and in PI-experienced patients. Few data concerning the determinants and the emergence of drug resistance in LPV/r-treated patients are available. In the LPV/r arm of a first-line ARV therapy protocol, all virological failures (VF) were shown to correspond to rebounds with wild-type HIV-1 (B. Bernstein, J. Moseley, D. Kempf, M. King, K. Gu, E. Bauer, and E. Sun,

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Abstr. 8th Conf. Retrovir. Opportunistic Infect., abstr. 453, 2001). A panel of viral isolates from patients failing therapy with other PI were used to show that 11 amino acid mutations in the protease were associated with a reduced sensitivity to LPV (7). The number of baseline mutations out of the cumulative number of these mutations (LPV mutation score) was shown to be predictive of the virological response to a regimen including LPV/r in PI-experienced, nonnucleoside HIV-1 reverse transcriptase inhibitor (NNRTI)-naïve patients (D. Kempf, S. Brun, R. Rode, J. Isaacson, M. King, Y. Xu, K. Real, A. Hsu, R. Granneman, Y. Lie, N. Hellmann, B. Bernstein, and E. Sun, 4th Int. Workshop HIV Drug Resist. Treatment Strategies, 12 to 16 June 2000, Sitges, Spain, abstr. 89, 2000). In this study, the overall virological response was important, since efavirenz (a NNRTI) was systematically coadministered, enhancing the efficacy of the ARV therapy. We thought it important to precisely identify the virological and pharmacological determinants of the virological response to LPV/r-containing regimens in the context of a salvage therapy in multiple-PI-experienced, frequently NNRTI-experienced patients.

MATERIALS AND METHODS

Study design. From May to November 2000, patients followed up at the Bordeaux University Hospital by the Aquitaine Cohort of the Groupe d'Epidémiologie Clinique du SIDA en Aquitaine and receiving LPV/r in the setting of the French Drug Agency temporary authorization for use (ATU) were prospectively enrolled into this study. Patients were PI experienced, and there were strict ATU entrance criteria (CD4 cells $< 200/\mu\text{l}$ and $\log_{10}$ copies of plasma HIV-1 RNA/ml > 4) when enrollment began, with a progressive enlargement to authorize the use of LPV/r in a larger PI-experienced population. At baseline of LPV/r therapy (month 0 [M0]) demographic data, prior and current ARV regimens, HIV-1 RNA, and CD4⁺ cell count were collected, as well as HIV-1 protease and reverse transcriptase (RT) gene sequences. In two patients, on treatment interruption at M0, preinterruption parameters measured 2 (for one patient) and 3 months (for the other) before beginning LPV/r were considered for the analysis. Patients were followed up at M1, M3, and M6 with viral load and CD4 measurements and additional HIV-1 genotype determination in case of VF, defined as plasma HIV-1 RNA level of ≥ 400 copies/ml.

Virological analyses. Plasma HIV-1 RNA was quantitated using the bDNA Quantiplex assay, version 3.0 (Chiron Bayer, Emeryville, Calif.). The RT and protease gene sequences were determined from plasma samples by the Agence Nationale de Recherches sur le SIDA (ANRS; Paris, France) consensus method (10) with a CEQ L sequencer (Beckman Coulter) as previously described (8). All individual ARV resistance mutations reported by the International AIDS Society-USA (IAS-USA) panel (<http://www.iasusa.org>) were considered. For each patient at baseline LPV/r an LPV mutation score was defined as the number of protease mutations out of the following 11 mutations: L10F/I/R/V, K20M/R, L24I, M46I/L, F53L, I54L/T/V, L63P, A71I/L/V/T, V82A/F/T, I84V, and L90M. The virology laboratory of Bordeaux participates to the ANRS quality control assessment of HIV-1 drug resistance sequencing (F. Brun-Vezinet, D. Descamps, V. Calvez, M. L. Chaix, J. Izopet, B. Masquelier, A. Ruffault, C. Tamalet, P. Dehertogh, C. Loveday, L. Perrin, D. Costagliola, and The ANRS Resistance Group, 5th Int. Workshop HIV Drug Resist. Treatment Strategies, 4 to 8 June 2001, Scottsdale, Ariz., abstr. 157, 2001).

Pharmacokinetic analysis. (i) **Data preparation and pharmacokinetic analysis.** Patient data files were created from observed data for LPV plasma concentrations by using the PASTRX program in USC*PACK PC clinical programs (R. Jelliffe, University of Southern California, Los Angeles, Calif.). Clinical, pharmacokinetic, and demographic data and treatment history relevant to the pharmacokinetic analysis were obtained. At entry and each follow-up visit, the patient filled in a questionnaire seeking information on the dates, times, and doses of nucleoside RT inhibitors (NRTI) and PI taken and recent over-the-counter medication used. Each individual provided one blood sample per visit: a predose trough sample (for trough LPV plasma concentration [$C_{\min}$]) or a postdose sample (maximum concentration of the drug in plasma [$C_{\max}$]) collected between 2 and 5 h after the dose taken during the visit. Blood samples for the pharma-

cokinetic study were drawn at M1 (steady state) and then at M3. For the analysis of possible relationships between pharmacokinetic and virologic parameters, areas under the plasma concentration-time curve over a 12-h dosing interval (LPV AUC₀₋₁₂) were used, with a cutoff value defined as the median LPV AUC₀₋₁₂ obtained in the study.

(ii) **Plasma sample analysis.** Plasma LPV/r concentrations were measured by a validated high-performance liquid chromatography method and UV detection. The lower and upper limits of LPV quantification were 0.05 and 50 mg/liter, respectively. For that study, assay correlation coefficients (20 analytical runs) exceeded 0.998 for LPV. Based on quality control samples, interday accuracy for the two analytes ranged from 99 to 101% of a known concentration and interday variability was $< 8\%$. In total, 59 blood samples were drawn at M1 versus 65 at M3, with 0.87 sample per patient at M1 and 0.96 sample per patient at M3.

(iii) **Pharmacokinetic model.** The pharmacokinetic analysis was performed by nonparametric method NPEM2 (R. Jelliffe), which considered only LPV concentrations. NPEM2 used a model having absorptive, central, and peripheral compartments. Parameters included K_a (absorption rate constant), V (volume of distribution), K_{cp} , and K_{pc} (constants between central and peripheral compartments), K_e (elimination rate constant), and F (fraction orally absorbed). A one-compartment model with first-order absorption and first-order elimination was used to fit the data. Assay variability was determined, and the absorption rate constant and the ratio of the volume of distribution to the fraction orally absorbed (V/F) were fixed. Individual K_e was calculated, and ratio of clearance (CL) to F was extrapolated from the equation $CL/F = K_e \times V/F$. Individual CL/F was used to determine individual exposure to LPV by calculating AUC₀₋₁₂ from the equation $CL/F = (\text{dose} \times F)/\text{AUC}_{0-12}$.

Statistical analysis. For comparison of qualitative variables, we used the Pearson chi-square or Fisher test and Student's test or the nonparametric Kruskal-Wallis test for quantitative variables. All variables associated with the outcome (VF at M3, defined as ≥ 400 copies of plasma HIV-1 RNA/ml) with a P value of < 0.25 in the univariate analysis were included in multivariate models by using a descending stepwise logistic regression. The variables significant at the 0.05 level were kept in the final models to study first the significance of the LPV mutation score and then the significance of individual protease mutations and pharmacokinetic parameters for the virological response. Four different models were used because of the colinearity between the LPV mutation score and individual mutations and the lower number of patients with pharmacokinetic measurements. Analyses were performed using Stata (College Station, Tex.) statistical software.

RESULTS

Baseline characteristics. Sixty-eight patients were included in the study. Their biological and clinical characteristics are described in Table 1. Most of the patients were multiple-PI and NNRTI experienced, with a high viral load at baseline. The HIV-1 genotypes for 66 patients were obtained, the other two patients having a viral load below 500 copies/ml at baseline. The prevalence figures of baseline protease resistance mutations, as reported by the IAS-USA panel (6) are summarized in Fig. 1A in the format of categories of the LPV mutation score. The mean ($\pm$ standard deviation [SD]) number of protease resistance mutations was 7 ± 3 (median, 7; range, 1 to 19), the mean LPV score was 5 ± 2 (median, 5; range, 1 to 9). The RT gene sequences for 65 patients were determined; the prevalences of the baseline RT resistance mutations are shown in Fig. 1B. The mean number of NRTI resistance mutations was 5 ± 2 (median, 5; range, 0 to 9), and the mean number of nucleoside-associated mutations (NAMS; formerly zidovudine resistance mutations; M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E) was 3 ± 1 (median, 3; range, 0 to 5). Ten patients had no NNRTI resistance mutation.

Description of the LPV/r-containing regimens and virological and immunological responses. The NRTI combined with LPV/r were zidovudine, didanosine, zalcitabine, lamivudine, stavudine, and abacavir in 5 (7%), 45 (67%), 7 (10%), 32 (48%), 28 (42%), and 18 (27%) of the 68 patients, respectively.

TABLE 1. Clinical and biological characteristics of the patients at baseline LPV/r therapy, Aquitaine Cohort, 2000 to 2001

Parameter	<i>n</i>	Value
Mean age (yr) (SD)	68	41 (9)
No. of men (%)	68	53 (78)
No. (%) in HIV transmission category	68	
Men who have sex with men		31 (45)
Intravenous drug users		14 (21)
Heterosexuals		14 (21)
Others or unknown		9 (13)
No. (%) in CDC ^a stage	65	
A		13 (20)
B		23 (35)
C		29 (45)
Mean CD4 cell count/ μ l, (SD)	44	174 (132)
Mean plasma HIV-1 RNA (log ₁₀ copies/ml) (SD)	68	4.6 (0.8)
No. (%) with previous antiretroviral treatments	68	
NRTI		68 (100)
Zidovudine		66 (97)
Didanosine		61 (88)
Zalcitabine		49 (72)
Lamivudine		67 (98)
Stavudine		66 (97)
Abacavir		36 (53)
NNRTI		61 (90)
Nevirapine		51 (75)
Delavirdine		4 (6)
Efavirenz		35 (51)
PI		68 (100)
Saquinavir		49 (72)
Ritonavir		47 (69)
Indinavir		58 (85)
Nelfinavir		55 (81)
Amprenavir		19 (30)
Mean no. of previous PI (SD)		3 (1)

^a CDC, Centers For Disease Control and Prevention.

Nevirapine and efavirenz were prescribed in eight (12%) and seven (10%) patients, respectively. LPV/r-containing regimens associated three drugs in 53 patients, four drugs in 13 patients, and five drugs in 2 patients. The patients exhibited a median decrease of plasma HIV-1 RNA of 1.16 (0.40 to 2.37) log₁₀ copies/ml at M3 (*n* = 62) and of 0.90 (0.42 to 1.80) log₁₀ copies/ml at M6 (*n* = 63) and mean increases of 64 (range, 35 to 107) CD4⁺ cells/ μ l at M3 (*n* = 48) and of 81 (range, 34 to 140) CD4⁺ cells/ μ l at M6 (*n* = 35). The proportion of patients with a plasma HIV-1 RNA below 400 copies/ml was 34% at M3 (*n* = 65) and 28% at M6 (*n* = 61). With a less strict definition (plasma HIV-1 RNA of <400 copies/ml and/or a decrease of plasma HIV-1 RNA of ≥ 1 log₁₀ copy/ml), the proportion of virological response reached 55% at M3 and 46% at M6. Between M0 and M3, four patients stopped LPV/r therapy and one died of an AIDS-related syndrome. Two patients stopped LPV/r between M3 and M6.

Pharmacokinetic results. Sixty-eight patients were included in this pharmacokinetic study. At pharmacokinetic steady

state, the mean LPV plasma concentrations and times to reach these concentrations at M1 and M3, respectively, were as follows: *C*_{min}, 3.88 $\pm$ 1.97 and 4.22 $\pm$ 2.66 mg/liter; *T*_{min} (time to reach LPV *C*_{min}), 12.19 $\pm$ 2.56 and 12.49 $\pm$ 1.99 h; *C*_{max}, 9.79 $\pm$ 5.09 and 9.10 $\pm$ 5.09 mg/liter; *T*_{max} (time to reach LPV *C*_{max}), 3.26 $\pm$ 1.42 and 2.29 $\pm$ 0.68 h.

The LPV exposures determined from the individual AUC₀₋₁₂ were 87 $\pm$ 21 and 77 $\pm$ 53 mg/liter $\cdot$ h at M1 and M3, respectively. The median LPV AUC₀₋₁₂ in the whole study was at 80 mg/liter $\cdot$ h and defined the cutoff value for comparisons. The mean calculated *C*_{min} values at M1 and M3 were 3.37 $\pm$ 0.86 and 2.93 $\pm$ 0.80 mg/liter, respectively.

Determinants of the response to LPV/r. The univariate analysis showed that the following were associated with a poor virological response (*P* < 0.250): the presence of baseline protease substitutions M46I, I54V, and V82A; an LPV mutation score >5; a large total number of protease mutations; a low LPV AUC₀₋₁₂; a low LPV *C*_{min} at M1 and M3; prior exposure to didanosine, abacavir, nevirapine, ritonavir, indinavir, or amprenavir; a high number of previous PI, a high number of treatment lines; and prior exposure to NNRTI. On the other hand, the coprescription of efavirenz, the presence of baseline protease mutation V77I, D30N, or N88D, an older age at inclusion, and the female gender were associated with a better virological response. The characteristics of the RT gene sequence (total number of NRTI and NNRTI mutations, number of NAMS) were not associated with the virological outcome at M3.

The median LPV AUC₀₋₁₂ values at M1 in patients with virological response and in patients with VF were 104 (interquartile range [IQR], 97 to 114) and 79 (IQR, 67 to 90) mg/liter $\cdot$ h, respectively (*P* < 0.0001). The median LPV *C*_{min} values at M1 in patients with virological response and in patients with VF were 4.01 (IQR, 3.76 to 4.23) and 3.08 (IQR, 2.65 to 3.81) mg/liter, respectively (*P* < 0.0001).

The results of the multivariate analysis of the determinants of the virological response, according to four different models, are shown in Table 2. In the first model, a higher number of prior PI, prior therapy with at least one NNRTI, and an LPV mutation score higher than 5 were associated with VF; on the other hand, the female gender and the presence of efavirenz within the LPV/r-including regimen were associated with a better virological response. Four of six patients who received efavirenz with LPV/r had a virological response, with a baseline NNRTI resistance genotype encoding the following mutational patterns: V106A, K101E plus Y181I, wild type, and Y181C plus G190A. The other two patients had VF, one with a G190A mutation and one with a Y181C at baseline.

To explain the protective effect of the female gender found in our analysis, we compared the different characteristics of the patients according to gender. No statistically significant difference was observed, but the median LPV *C*_{min} at M1 tended to be higher in women than in men (3.93 versus 3.23 mg/liter; *P* = 0.08).

The second model was constructed to evaluate the role of individual protease mutations. The presence of the I54V mutation was independently associated with a poor virological response at M3. The I77V mutation was associated with a better virological response at M3.

The third model included pharmacokinetic parameters and

FIG. 1. (A) Mutations in the HIV-1 protease in 66 patients at baseline therapy with LPV/r. Protease mutations are those reported by the IAS-USA panel (<http://www.iasusa.org>). LPV mutations, mutations included in the LPV mutation score, i.e., the number of baseline protease mutations out of 11 possible mutations: L10F/I/R/V, K20M/R, L24I, M46I/L, F53L, I54L/T/V, L63P, A71I/L/V/T, V82A/F/T, I84V, and L90 M; other R mutations, mutations not included in the LPV mutation score; % of patients, percentage of patients with the corresponding mutation at baseline LPV/r. (B) Mutations in the HIV-1 RT in 65 patients at baseline LPV/r. NRTI and NNRTI resistance mutations are those reported by the IAS-USA panel.

the detailed description of prior PI use. Prior treatment with ritonavir or indinavir was associated with a worse response; a higher LPV AUC₀₋₁₂ at M1 was associated with a better response. In a model including the number of prior PI but not the details of the prior PI, LPV AUC₀₋₁₂ was still a predictive factor (data not shown). The fourth model shows that higher calculated LPV C_{min} at M1 was also associated with a better response. The LPV mutation score remained predictive of the virological response in all the multivariate models. The virological response according to the LPV mutation score is shown in Fig. 2, with a reduced response in the patients with six or

more mutations, particularly using a strict definition of the virological response.

Evolution of the protease genotype in patients failing on LPV/r. The protease gene sequence could be determined at M3 and/or M6 on an LPV/r-containing regimen for 48 patients without virological response at these points of follow-up. We determined the variations for any amino acid between baseline and M3 and M6. Fifteen patients exhibited no change between M0 and M3 or M6. For the other 33 patients, at least one residue was changed. As shown (Table 3), different PI resistance mutations appeared on LPV/r, corresponding to muta-

TABLE 2. Multivariate analysis of the factors associated with VF under LPV/r therapy, Aquitaine Cohort, 2000 to 2001

Variable	Model 1 (<i>n</i> = 67)		Model 2 (<i>n</i> = 67)		Model 3 (<i>n</i> = 66)		Model 4 (<i>n</i> = 65)	
	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>
Gender (female)	0.09 (0.01–0.59)	0.012	0.12 (0.02–0.66)	0.015	0.03 (0.002–0.38)	0.002	0.05 (0.004–0.57)	0.016
No. of prior PI	3.28 (1.09–6.88)	0.002	2.38 (1.27–4.49)	0.007		NI	4.18 (1.52–11.46)	0.005
Prior NNRTI	10.62 (1.09–103.21)	0.042		NS	96.23 (3.13–2962.91)	0.009	33.61 (1.55–727.51)	0.025
Prior ritonavir		NI		NI	14.03 (1.4–140.47)	0.025		NI
Prior indinavir		NI		NI	73.62 (2.59–2090.77)	0.012		NI
Association with efavirenz	0.53 (0.004–0.64)	0.021	0.05 (0.005–0.47)	0.009		NS	0.03 (0.001–0.76)	0.033
LPV mutation score > 5	10.11 (1.86–54.87)	0.007		NI	22.33 (2.01–248.10)	0.011	12.80 (1.36–120.44)	0.007
154V at baseline		NI	3.60 (1.29–9.99)	0.014		NI		NI
177V at baseline		NI	0.28 (0.10–0.77)	0.014		NI		NI
LPV C _{min} (M1)		NI		NI		NI	0.09 (0.012–0.090)	0.016
LPV AUC _{0–12} (M1)		NI		NI	0.91 (0.86–0.97)	0.007		NI

^a NI, not included in the model; NS, not significant (*P* > 0.05). The LPV mutation score is the number of mutations out of the 11 following protease mutations: L10F/I/R/V, K20M/R, L24I, M46I/L, F53L, I54L/T/V, L63P, A71L/V/T, V82A/F/T, I84V, L90M. OR, odds ratio; CI, confidence interval.

tions included or not included in the LPV mutation score. Interestingly, one primary resistance mutation (I50V) formerly described to be associated with resistance to amprenavir, was selected in four patients, three of whom had no prior amprenavir therapy. Other changes were selected at 13 different positions not involved so far in resistance to PI, with low frequencies (one or two patients for each amino acid); for four patients only changes within these 13 positions were detected. In 38 patients without virological response at M3 and M6 and with a calculated LPV AUC_{0–12} at M1 or M3, the viral evolution in the protease (with at least one additional PI resistance mutation at M3 and/or M6) occurred in 4 of 12 (33%) patients with low (<80 mg/liter · h) LPV AUC_{0–12} and in 22 of 25 (88%) patients with correct or high LPV AUC_{0–12} (*P* < 0.001).

DISCUSSION

In this study, we described the determinants of the virological response to LPV/r-containing regimens in PI-experienced patients. The response to therapy in our study, using strict criteria (plasma HIV-1 RNA < 400 copies/ml at M3), was lower than in the initial study (Kempf et al., 4th Int. Workshop HIV Drug Resist. Treatment Strategies, 2000, Sitges, Spain, abstr. 89) of the response to LPV/r in PI-experienced patients. This could be explained by the characteristics of our population: highly pretreated and NNRTI and multiple PI experienced, with a high number of PI resistance mutations at baseline. Nevertheless, the previously constructed LPV mutation score was shown to be predictive of the virological response in our multivariate analysis, with a higher risk for VF with a score at six or more mutations. Similar data were obtained in another study (V. Calvez, I. Cohen-Codar, A. G. Marcelin, E. Guillevis, J. Isaacson, R. Rode, B. Bernstein, E. Sun, D.

FIG. 2. Virological response to LPV/r-containing regimens at M3 according to the LPV mutation score (*n* = 65). <400 copies/ml, patients with plasma HIV-1 RNA levels of <400 copies/ml at M3; <400 c/ml or $-1\log_{10}$, patients with plasma HIV-1 RNA levels of <400 copies/ml at M3 and/or with a decrease of plasma HIV-1 RNA by $>1\log_{10}$ unit between M0 and M3; LPV mutation score, number of baseline protease mutations out of 11 mutations: L10F/I/R/V, K20M/R, L24I, M46I/L, F53L, I54L/T/V, L63P, A71I/L/V/T, V82A/F/T, I84V, and L90M.

TABLE 3. Amino acid mutations selected in the HIV-1 protease in 48 patients on LPV/r-containing regimens, Aquitaine Cohort, 2000 to 2001

Mutation(s) ^a	No. of patients
M46I	9
I54V/A/M	8
L33F.....	6
V82A/T/I	5
K20M/R	6
L10I	4
A71V	4
M36I.....	4
150V.....	4
F53L/W	3
147V.....	3
V77I.....	3
N88D/T.....	2
L63P, G73S, I84V, L90M	1

^a Mutations are PI resistance mutations listed by the IAS-USA panel (www.iiasusa.org). Mutations included in the LPV mutation score are in boldface.

Kempf, and J. P. Chauvin, 5th Int. Workshop HIV Drug Resist. Treatment Strategies, 2001, Scottsdale, Ariz., abstr. 82, 2001), suggesting that the LPV mutation score could be relevant for optimizing the use of LPV/r in salvage therapy. We also explored the implication of specific mutations in the response to LPV/r. The presence of the L54V protease mutation at baseline was associated with a higher risk of VF. Although this mutation can be considered a secondary PI resistance mutation, it seems to play an important role in resistance to LPV. In a study using correlations between reduced phenotypic sensitivity to LPV and the protease gene sequences from clinical isolates (P. R. Harrigan, C. Van Den Eynde, and B. A. Larder, Scottsdale workshop, abstr. 49, 2001) the L54V mutation was associated with the highest decrease in susceptibility to LPV/r. This mutation also belonged to the mutations appearing on LPV/r in the followed-up patients without optimal virological response in our study. These findings are consistent with the hypothesis that the addition of the I54V mutation on a background of previously established PI mutations enables the virus to acquire high-level resistance to LPV without impairing its replication capacity.

Our finding that the use of efavirenz reduced the risk of treatment failure has to be interpreted with regard with the baseline pattern of NNRTI resistance mutations in patients who received efavirenz and had a virological response. The absence of mutation K103N, which is frequently found in efavirenz-resistant isolates, could explain a residual activity of efavirenz. A potential interpretation of these data, despite a limited number of patients, is that efavirenz could contribute significantly to the virologic success of a regimen, even in NNRTI-experienced patients, when administered as part of a sufficiently potent regimen.

The observed individual LPV pharmacokinetic parameters such as C_{min} , T_{min} , C_{max} , and T_{max} seemed to be stable when the LPV pharmacokinetic was in steady state. This observed stability between M1 and M3 should enable us in the future to monitor individual LPV plasma concentrations and to adapt LPV/r dosages when necessary. All our pharmacokinetic results were similar to previously reported data (R. Bertz, W. Lam, and S. Brun, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 327, 1999). However, the C_{min} and AUC_{0-12} levels found in the group of virological responders (about 4 mg/ml and 100 mg/ml · h, respectively) suggest that higher LPV concentrations than previously described in first-line drug-treated patients could be necessary to be active on the first-available-PI-resistant viruses. A lower LPV individual exposure (AUC_{0-12}), as well as lower LPV trough concentrations at M1, were associated with a higher risk of VF at M3; interestingly, the predictive value of the baseline LPV mutation score was strengthened in the multivariate models including the pharmacokinetics parameters, suggesting that both genotypic and pharmacokinetic follow-up are of interest for the monitoring of LPV/r-containing salvage regimens.

One striking finding was the protective effect of the female gender found in the multivariate analysis. The higher LPV C_{min} found in women could partially explain this effect. In a recent study, a lower clearance of saquinavir, resulting in an higher exposure to this drug, was found to be related to the female gender (R. C. Brundage, E. Acosta, R. Aubrich, D. Katzenstein, R. Culick, and C. V. Fletcher, Abstr. 9th Conf.

Retrovir. Opportunistic Infect., abstr. 779, 2002). Although no data concerning the adherence to therapy, a putative confounding factor, were available in our study, a similar difference in LPV clearance could account for the observed effect of the female gender.

In a subset of patients with uncontrolled plasma HIV-1 RNA on LPV/r, we found evidence for the selection of additional PI resistance mutations. Neither a specific resistance pattern nor any new and frequent mutation could be inferred from these data, which are consistent with the evolution toward high-level resistance to LPV through the accumulation of greater numbers of changes in the protease. However, the appearance of the I50V mutation in four patients was of particular importance, since it was recently shown to mediate the emergence of viruses cross-resistant to both LPV and amprenavir (J. G. Prado, J. G., T. Wrin, J. Beauchaine, L. Ruiz, C. J. Petropoulos, B. Clotet, R. D'Aquila, and J. Martinez-Picado, Scottsdale workshop, abstr. 67, 2001), precluding the possibility of use of the latter drug in patients on LPV/r in which this mutation is selected. These data also suggest that further studies should be done in order to evaluate the effect of the I50V mutation on the response to an LPV/r-containing regimen in an amprenavir-experienced population.

The correlations between pharmacokinetic parameters and the evolution of the HIV-1 protease in the patients on LPV/r without a complete virological response suggested that low plasma LPV concentrations favored the replication of unchanged PI-resistant viruses. In other patients, VF could occur despite normal LPV concentrations, with the selection of additional PI resistance mutations, leading to broader resistance patterns.

In conclusion, our results showed that, in multiple-PI-experienced patients, the virological response to LPV/r-containing salvage regimens can be predicted by the LPV mutation score, even if specific mutations can play a major role in LPV resistance. LPV plasma levels should then be optimized by using pharmacokinetics measurements in order to avoid the evolution of the virus toward high-level resistance to LPV and to other PI.

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